# Effects of frozen storage on quality of Atlantic mackerel (*Scomber scombrus*) caught in Icelandic Waters.

Improving mackerel products in order to increase their value

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May 2015

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# Effects of frozen storage on quality of Atlantic mackerel (*Scomber scombrus*) caught in Icelandic Waters.

Improving mackerel products in order to increase their value

Thesis for the degree of Master of Science in A thesis in fulfilment of the requirement of the degree of Master of Science in Food Science (90 ECTS units)


UNIVERSITY OF ICELAND
SCHOOL OF HEALTH SCIENCES
FACULTY OF FOOD SCIENCE AND NUTRITION

## DECLARATION

I hereby declare that this thesis is based on my own observations, is written by me and has not been in part or as a whole 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 gráðu.



Bergen 28.05 2014

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Andri Þorleifsson

# ABSTRACT

Mackerel (*Scomber scombrus*), has in the resent years, become one of the most valuable fish spies in Iceland. Utilizing this recourse effectively has create many challenges for Icelandic fish processors. One of those challenges is stopping or diminishing the spoilage rate during frozen storage. The main objective of this project was to study seasonal variation and the influence of frozen storage (-27°C for up to 4 months) on quality degradation of headed and gutted (H/G) mackerel with emphasise on lipid oxidation and hydrolysis. Mackerel samples were collected five times, during the fishing season, from late June to beginning of September, all from the vessel Heimaey VE-1. Quality inspections and frozen storage of the samples were done at fish processing plant belonging to Isfelag HF. Quality inspections of the raw material and final products were carried out using the same methods used at this plant to reflect common practices in the Icelandic processing plants. Samples were stored in cold storage (-27°C +/- 2°C), used by Isfelag for all its products.

The biological stability of initial raw material was evaluated in term of quality parameters, TVB-N measurements conducted to evaluate the freshness and proximate content measured to clarify the seasonal variation of the mackerel. To explore the oxidation stability of the mackerel, traditional methods including peroxide value (PV), thiobarbituric acid reactive substance (TBARS) and fluorescence method were used to determine the primary, secondary and tertiary lipid oxidation products respectively. Lipid hydrolysis (free fatty acid) was also measured to evaluate enzymatic activity.

Results showed, as confirmed by numerous other analyses, poor condition of the mackerel early in the season. Later on in the season biological stability improved considerable. Peroxide values diminished during 4 month frozen storage for all samples except the one from the beginning of the season, late June. FFA, TBARS and fluorescene increased in all samples during 4 months frozen storage. Oxidation rate was higher in fish with higher lipid content (July/August) indicating that spoilage rate of the mackerel was more influenced by lipid content than biological stability during frozen storage at (-27°C +/- 2°C).

## ACKNOWLEDGEMENTS

This study was conducted at local laboratorium Rannsóknarþjónusta Vestmannaeyja, Isfelag hf. in Vestmannaeyjar and Matis ltd. Icelandic Food and Biotech R&D (Matís ohf. Reykjavik).

The raw material came from Isfelag Vestmannaeyja hf. Analyses were made at Rannsóknarþjónusta Vestmannaeyja and Matis ohf. I thank them all.

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# 1. INTRODUCTION

Atlantic mackerel (*Scomber scombrus*) is a valuable pelagic fish found on both sides of the Atlantic Ocean. It´s been utilized for both fish meal and human consumption trough out the years (FAO 1983). Total world catches of Atlantic mackerel was 910.000 tons in 2012. Average catches from 1966-2012 were about 738.000 tons annually. Norway and UK have traditionally been the largest catchers of Atlantic mackerel, but in recent years Iceland and Faroe Island have increased their catches dramatically (FAO 2013).

Numerous products have being developed using Atlantic mackerel as a raw material. Consumption of mackerel products takes place all over the world, but the main markets are Japan, EU, Eastern European countries and Africa. Those markets differ considerably regarding requirements and product specifications. The Japanese market wants high quality large mackerel with firm texture and without any texture damages. In Eastern Europe and EU smoking and canning of mackerel is common and the requirements for raw material quality not as strict as in Japan. Africa accepts different quality. The price differs very much between these markets in relation to quality. The most lucrative market is Japan who prefers mackerel caught in autumn and winter months when the texture of the muscle is firm and minimal feed damages (Pelagia 2015). This does not correlate with the fishing season in Iceland and therefore Icelandic processors have to concentrate on other markets (Steinthorsdottir 2009). In the year, 2005 large schools of Atlantic mackerel began to migrate to Icelandic water for feed during the period of June to September. At first, small amount of the mackerel was caught in Icelandic fishing grounds as a by catch from herring fishing. In 2007, Iceland vessels started systematic fishing for mackerel, mostly for fishmeal production. In the beginning, it was free fishing without any quota regulations. The fishing increased rapidly in the following years and in 2009, the government put a limit on the catching. From 2010, the quota has been around 130.000 tons. There are also restrictions stating that over 70% of the catch has to be for allocated to human consumptions (Stjornartidindi 2013). The fish is primarily frozen in blocks whole or headed and gutted (H/G), as raw material for further processing in Eastern Europe, Asia and Africa were it is mainly smoked, fried, minced or canned. Some of the mackerel caught late in the season has reach the standard set by Japanese buyers and been exported to Japan. Fishing of mackerel in Iceland takes place when the biological stability of

the fish is poor due to seasonal variations in lipid content (Huss 1995). The fish migrates to Icelandic waters right after spawning with very low lipid content. It starts heavy feeding resulting in rapid increase in lipid content. It store the lipid in the muscle something that influence the texture in negative way (Sveinbjornson et. al. 2008; Hagstofa 2014; Arason *et. al.* 2010).

Processing of mackerel products has become very important for the Icelandic fishing industry. Export value of mackerel products were about 157, 3 million USD, in 2013 making mackerel one of the most valuable fish spies in Iceland (Hagstofa 2014).

Mackerel is a highly perishable commodity recording considerable losses in quality before consumption. Many factors influence spoilage of mackerel. Ambient temperature, age of raw material before processing, processing- and storage conditions. Biological factors like seasonal variations in lipid content and sea temperature. Storage time and temperature are the major factors implicated in the loss of quality and shelf life of fatty fishes like Atlantic mackerel (Huss 1995).

The freezing of fish is an effective way of long-term preservation. During frozen storage, spoiling rate diminished. For fatty fish like mackerel, controlling the lipid oxidation is vital. Best way of doing that is by freezing the fish before damages begins and keeping the temperature as low as possible from catching until final storage (Huss 1995). It is important to understand how freezing rate and freezing time alter the quality of H/G mackerel.

# 2. LITERATURE REVIEW

## 2.1 THE MACKEREL

### 2.1.1. BIOLOGY OF MACKEREL

Atlantic mackerel (*Scomber scombrus*) is a pelagic fish belonging to the family of scombridae. It can be found in the northeast Atlantic from Norway to Morocco and the Canaries, and in the Mediterranean and Black Seas. In the North West Atlantic, it occurs from Labrador to North Carolina (FAO 1983). One characteristics of mackerel are sleek, compact and streamlined bodies who is designed for fast swimming. Male and female mackerel have very similar growth rates (Scott and Scott 1989). The fish is a powerful swimmer who lives for most parts in large groups offshore. It can be 25 years old and the largest individuals can be 60 cm long and weighing about 3 kg. (Ástþórsson *et. al*. 2010). The mackerel has the habit of gathering in dense schools of many thousands individuals (Bigelow and Schroeder 1953). Mackerel feeds on variety of pelagic animals, mostly planktonic crustaceans, fish eggs, and fish juveniles (Jónsson 1983).

Atlantic mackerel reaches sexual maturity at the age of 2 to 3, then around 30 cm long (Ástþórsson *et. al*. 2010). Mackerel are a swift-moving fish, swimming with very short sidewise movements of the rear part of the body and of the powerful caudal fin. When caught they beat a rapid tattoo with their tails on the bottom of the boat until exhausted. In addition, they require so much oxygen for their vital processes that when the water is warm (hence its oxygen content low) they must keep swimming constantly, to bring sufficient flow of water to their gill filaments, or else they die (Bigelow and Schroeder 1953). There is no swim bladder in the Atlantic mackerel (FAO 2013).

The Atlantic Mackerel is typically an open ocean fish with voracious feeding habits. They travel in schools that often contain thousands of fish. The swift swimming mackerel has a streamlined body and swims at high speeds for extended periods searching for food. All individuals within a specific school tend to be the same size. Since cruising speed increases significantly with age and size, scientists believe that conformity of body size within a specific school is necessary to allow all fish to maintain identical swimming speeds (FAO 2013).

Mackerel do not resort to any particular breeding grounds, but shed their eggs wherever their wandering habits have chanced to lead them when the sexual products ripen. It follows from this, and from the fact that mackerel vary so widely in abundance over periods of years that the precise localities of greatest egg production expected to vary from year to year, depending on the local concentrations of the fish (Bigelow and Schroeder 1953)

The mackerel is a moderately prolific fish; females of medium size may produce as many as 400,000 to 500,000 eggs in the aggregate, according to various estimates, with 546,000 reported for one weighing 17/8 pounds. However, it is seldom that as many as 50,000 are set free at any one time, and often many fewer, for the members of a given school spawn over a considerable period. The eggs are 0.97 to 1.38 mm. in diameter, with one large oil globule, and drift suspended in the water, chiefly shallow sea than the 5-fathom level. The rate of development depended by the temperature of the water. Incubation takes about 150 hours at 54° (12,2°C); 115-95 hours at 57°-61° (13,9°C – 16,1°C); about 70 hours at 64°-65° (17,8°C-18,3°C); and about 50 hours at 70°(21,1°C); with normal development limited to temperatures between about 52° (11,1° C.) and 70° (21,1° C.) (Bigelow and Schroeder 1953).

### 2.1.2 DISTRIBUTION

The Atlantic mackerel occurs from the northeast coast of USA, up to Newfoundland Island on the west side of the Atlantic Ocean (Figure 2.1). On the eastern side it is found off Morocco, in the Mediterranean sea and all the way up to the Barents Sea, although only occasionally. Three stocks are in the northeast Atlantic. The southern stock in Spanish and Portuguese waters, the western stock in the Bay of Biscay and around Ireland and the third stock spawns in the North Sea (Ástþórsson et. al. 2009).

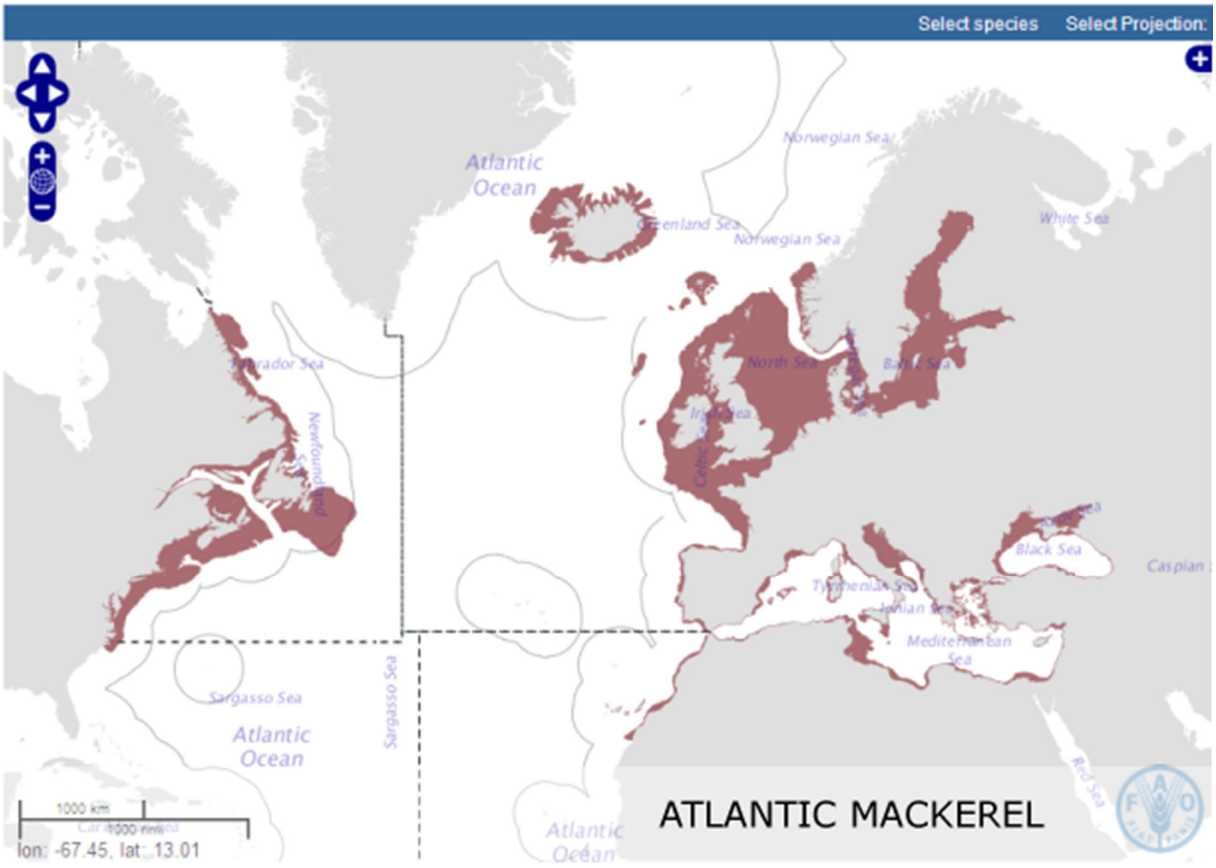



**Figure 2.1: Distribution of Atlantic mackerel (FAO 2015)**

Mackerel spawn off the American coast from the latitude of Cape Hatteras to the southern side of the Gulf of St. Lawrence. The spawning area covers almost the entire breadth of the continental shelf southward from Cape Cod, but confined more closely to the vicinity of the coast thence northward. Available data point to the oceanic bight between Chesapeake Bay and southern New England as the most productive area, the Gulf of St. Lawrence as considerably less so, and the Gulf of Maine and coast of outer Nova Scotia as ranking third (Bigelow and Schroeder 1953). The Atlantic Mackerel (Europe) consists of tree stocks in three spawning grounds, south, west and north. The south spawning-ground is located outside the cost of Spain and Portugal. The west spawning-ground is located in Biscay bay and off the west coast of Ireland and UK. The eggs from south and west spawning grounds mixes up and therefore difficult to distinguish between them. The third spawning-ground is the north spawning-ground is located in the middle of the North Sea and north of Skagerrak (Ásthórsson et. al. 2009)

The mackerel (in America) spawns in spring and early summer. As it does not commence to do so until the water has warmed to about 46° F. (7,8° C.), with the chief production of eggs

taking place in temperatures of, say, 48° to 57° (8,9°C-13,9°C), the spawning season is progressively later, following the coast from south to north. Thus, the chief production starts in Chesapeake Bay in the middle of April and ends in the southern side of the Gulf of St. Lawrence, where eggs have been taken from early June to mid-August (Bigelow and Schroeder 1953).

The Mackerel on the East side spawns from February to July depending on areas. In the south in April-May, in the west area May-June and in the north in June. When spawning occur the temperature on the surface of the ocean is lower than when its peeks. Therefore the spawning occurs, most often, in deeper waters at the beginning of the spawning season (Iversen 2004). When spawning is finished, the mackerel travels to look for food. Mackerels belonging to the south- and west stocks travel north to the Norway Sea and North Sea. South of Faroe Islands the main schools divides into to one that goes east of the Island and one that goes west of the Islands. The one that goes west is probably the one found in Icelandic waters. The one that goes east divides and goes to Northern North Sea and south-west Norway Sea (Ástþórsson *et. al.* 2009, FAO 2011)

The mackerel does not spawn in Icelandic waters, as far as we know, but migrates there occasionally throughout the country. It seems clear from archives that this happens regularly as large amount of mackerel were reported for many years in a row around 1900, and during the warm period from 1926 to 1945 and sporadically in between and after. It is also clear that it is now mass migrating into the Icelandic EEZ due to the current warm oceanic conditions (Ástþórsson et. al 2009). The mackerel in Icelandic waters is originated from the East Atlantic Ocean.

### 2.1.3 FISHING METHODS FOR MACKEREL

Most mackerel fisheries target large shoals offshore using mid water trawls[1] (Figure 2.2), pair trawls or purse seines. The adoption of dual and triple frequency sonar technology on some vessels has enabled skippers to differentiate between pelagic species. In inshore areas, small vessels use hand lines and gillnets, including a substantial Spanish fleet (4,950 vessels in 2009) and hand line fleets off the South West coast of England and coast of Scotland. Most mackerel caught in European waters, including Icelandic waters, are landed fresh, chilled with ice, using

[1] Mid-water trawls are used in large scale Icelandic mackerel fishing.

mixture of ice and seawater (CSW) or refrigerated seawater (RSW), to shore processors, although some of the large freezer vessels process and pack their catch at sea (Seafish 2012). Large-scale fishing of mackerel takes for the most parts place by large trawlers who haul for about 2-6 hours and pump carefully from the trawl bag into refrigerated tanks.

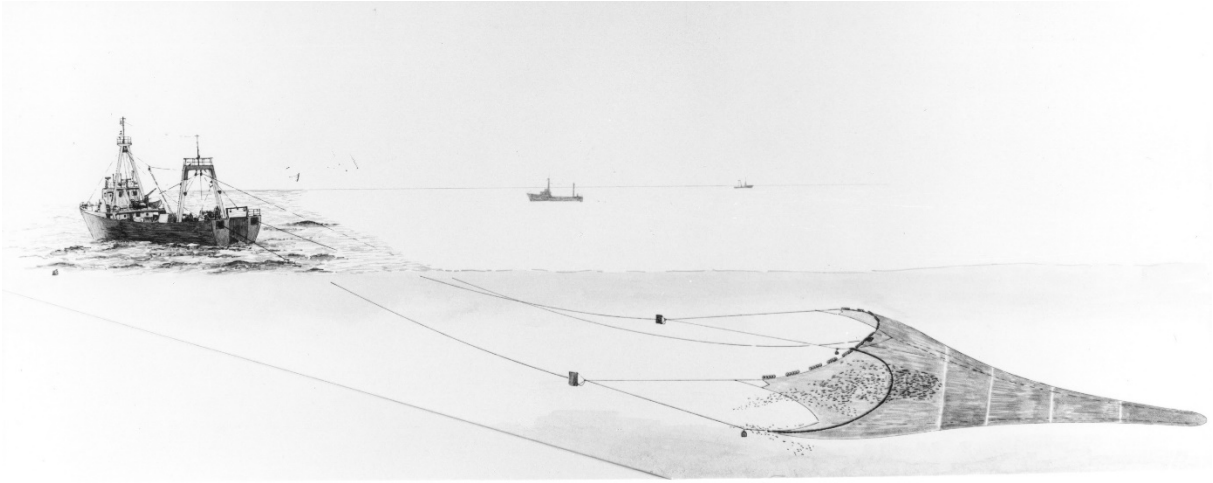


**Figure 2.2: Mid water trawl for mackerel fishing (Fisheries and Oceans Canada 2013).**

After catching, the fish requires immediate chilling to lower body temperature and slow rigor mortis, enzymatic activity and hence bacterial degradation (Nunes and Morâo de Campos 1992).

The raw materials in this study were taken from vessels using RSW system. In a standard RSW system, the system is fed via the water intakes in the hull. After the system has been filled, the valve is closed and the RSW pump circulates the seawater in the system through a filter to the evaporator and back to the tank through manifolds in both the bottom and top of the tank.

Figure 2.3 shows expected chilling process of a RSW system. In the first period ($\tau 1$), the water (general thumb rule is 1/3 of the total tank volume) in the storage tank is pre-chilled from temperature T1 to T3. In the second period ($\tau 2$), fish is dumped into the pre-chilled storage water. This raises the temperature up to an average between the fish and the chilled seawater (temperature of the mixture, T2). In the third period ($\tau 3$), the temperature is lowered to the target temperature and maintained there until the fish are landed. The length of the third period depends on the capacity of the refrigeration system and fished quantity (Thorsteinsson *et. al.* 2003).

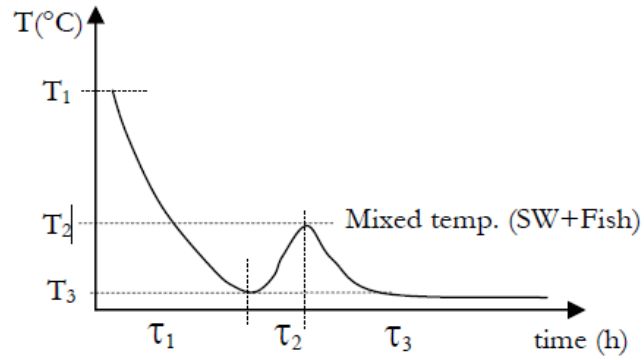



**Figure 2.3: Expecting chilling down figure for RWS system (Thorsteinsson *et. al.* 2003)**

According to SINTEF (2012) the Mackerel should be cooled down as quickly as possible down to 0 °C when entering the fish tank, down to 3°C at least six hours after being pumped in the tank and down to 0°C within 16 hours. When landed the mackerel should be about -1°C to -1,5°C in order to prevent any spoilage.

### 2.1.4 PRODUCTION OF HEADED AND GUTTTED MACKEREL (H/G)

In Figure 2.3, production flow of H/G mackerel is shown. This flowchart is from Isfelag hf.[2] Even though each producer has its own characteristics regarding the production flow, the principles are generally the same. Mackerel is pumped during landing from the chilled storage tank on board the fishing vessel to a grader (style grader) were it is size graded into two or three different sizes according to Product Specification from the buyer. From the grader the mackerel goes into tub with slurry ice (-2 °C), containing about 400 kg. fish and 100 litres slurry ice. According to Rodriguez *et. al*. (2004), the two main features of slurry ice are its faster chilling rate, deriving from its higher heat-exchange capacity, and the reduced physical damage caused to seafood products by its microscopic spherical particles, as compared with flake ice. The overall covering of the fish surface by the slurry ice mixture also protects the fish from oxidation by preventing the action of oxygen. The tubs, containing mackerel in slurry ice, goes to the processing plant. At the processing plant, the tubs are stored in chilled room (1-2°C) for 0-10hours before processing. Then the mackerel is headed, the viscera sucked out and the belly part cleaned through the mouth of the fish. This method obviates the need to open the belly, but it makes it difficult to sure efficient cleaning of the fish belly. When gutting

[2] Isfelag hf. Is an Icelandic fish processing company specialized in production of products from pelagic fish species

fish, it is important to thoroughly wash it to remove traces of blood and debris, and to wash bacteria and intestinal content out of the gut cavity, skin, and gills of the fish (Borderias and Sánchez-Alonso 2011). The H/G mackerel is then packed in plastic polyethylene bags, about 11 kg and about 500 ml of seawater is added for faster freezing. The bags go into plate freezers for about 4 hours setting the temperature down to about -30°C. It is important that the processing time is as short as possible to prevent fluctuations in temperature of the fish.

As shown on the diagram (Figure 2.4), the temperature in the mackerel changes during the process. During optimal conditions the processing time itself is about 10 minutes that is from the time the mackerel goes through heading and gutting and until it is packed in plastic bag and placed in the plate freezers. Freezing in plate freezers takes about 4 hours or until its core temperature reach -30°C. After casing, the product is stored at -27°C until they are exported. During transportation to the market, the temperature remains at about -25°C. However, the customer often stores the mackerel at -18°C. Mainly because on packaging is written store below -18°C.

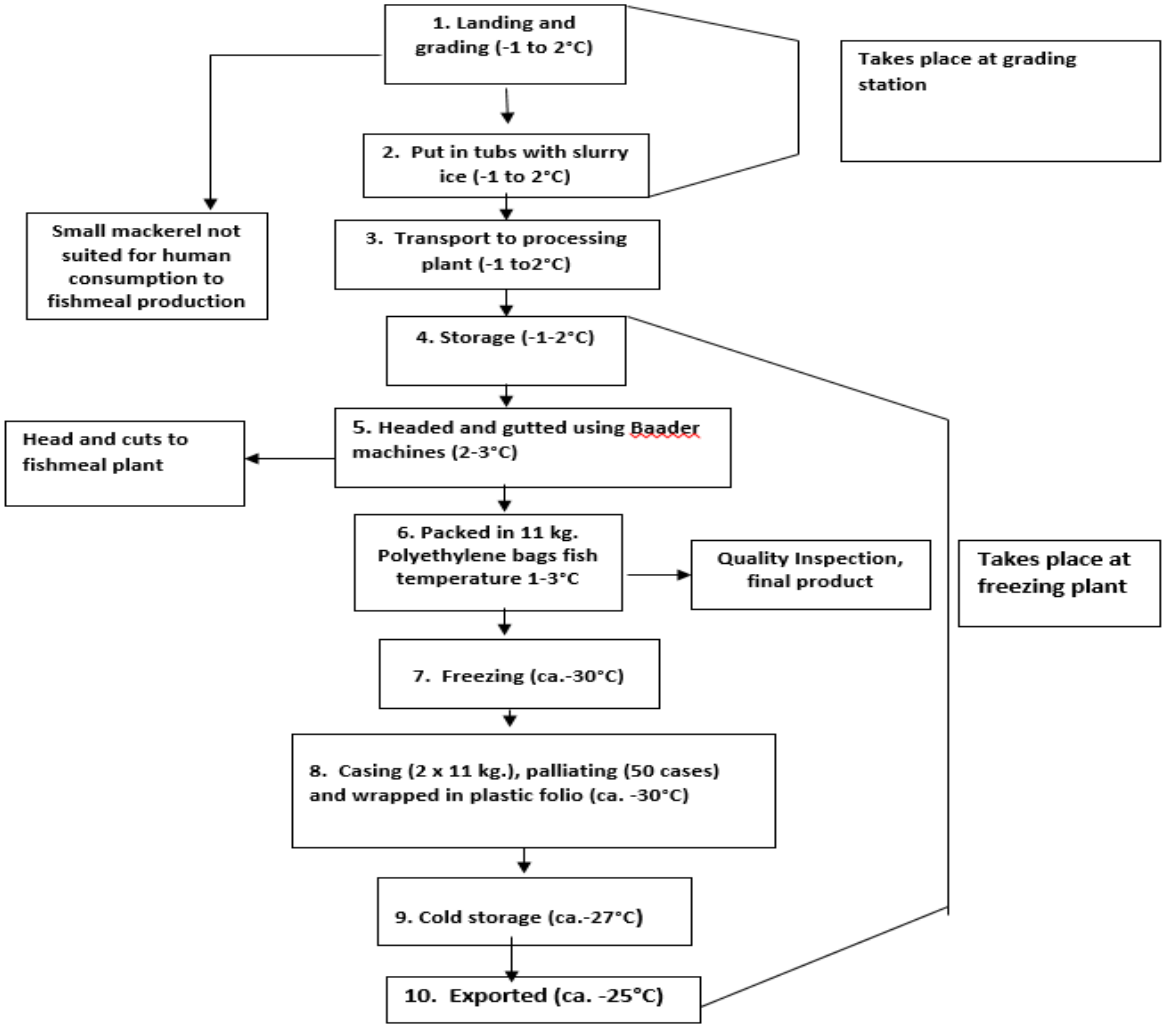



**Figure 2.4: Flow diagram of mackerel production at Isfelag and the temperature flow during the production.**

## 2.2 SUPPLY OF ATLANTIC MACKEREL

Total world catches of Atlantic mackerel was 910.000 tons in 2012. Average catches from 1966-2012 were about 738.000 tons annually. Norway and UK have traditionally been the largest catchers of Atlantic mackerel, but in recent years Iceland and Faroe Island have increased their catches dramatically (FAO 2013). Figure 2.5 shows annually catches of Atlantic mackerel from 2002 to 2012 in the whole world and Europe. European nation are responsible for most of Atlantic mackerel catches and the increase in catches, in recent years, are mainly in Europe.

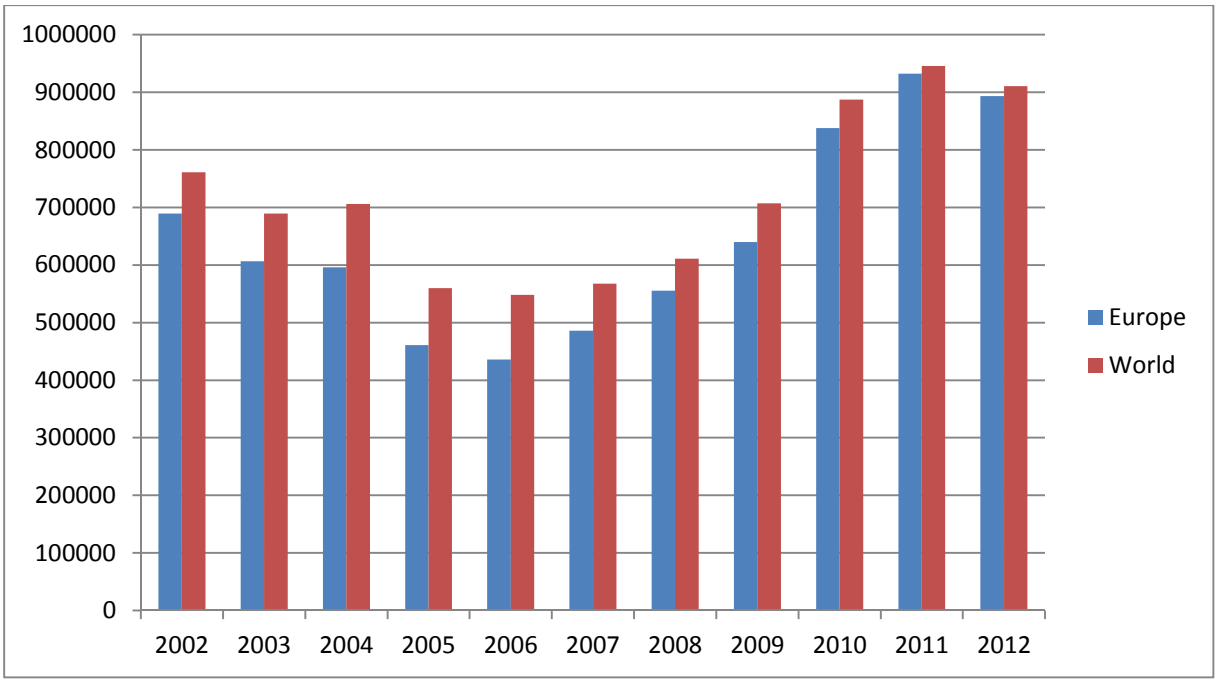



**Figure 2.5**: **World catch of Atlantic mackerel 2000-2010 (FAO 2014)**

Traditionally UK and Norway are the main fishing nations of Atlantic mackerel in Europe, and also in the world. Figure 2.6 shows fishing of Atlantic mackerel divided by nations from 2008 to 2012. Norway is the leading fishing nation followed by UK. Iceland and Faroe Islands have increased its fishing since the mackerel started to migrate into their fishing areas.

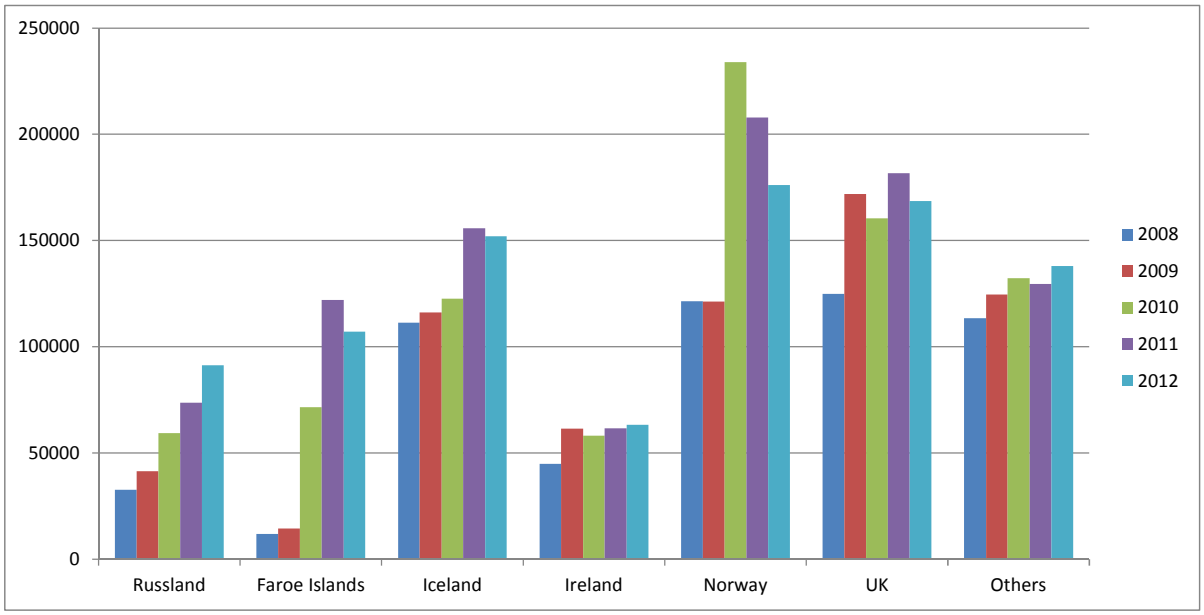



**Figure 2.6: Fishing of Atlantic mackerel in Europe divided by nations, 2008-2012 (FAO 2014)**

## 2.3 MARKETS FOR ATLANTIC MACKEREL

Since 2009 an increase of about 200.000 tons of Atlantic mackerel has been caught annually and has to be allocated into the market.

Traditionally the fishing season in Europe has been during the winter months when lipid content and texture is optimum for high paying markets like Japan. Iceland and Faroe Islands are only capable of catching the mackerel when it is in their Waters, during the summer months when the fish has just finished spawning and had low lipid content and is vulnerable for damages. This has been a big challenge for Icelandic- and Faroese processors. Due to the condition of the mackerel it has been impossible for them to enter markets that are willing to pay higher price for quality, like the Japanese market. Export to less attractive markets like east Europe and Africa has been the reality of Icelandic and Faroese mackerel. In Figure 2.7 and Figure 2.8 allocation of mackerel products from Icelandic and Norwegian processors are shown. There is almost no export from Iceland to Japan, but Norway exports large amounts of mackerel to Japan. Additionally Norway exports to EU, something that Icelandic processors are not allowed doing due to disagreement about how to regulate the mackerel fishing.

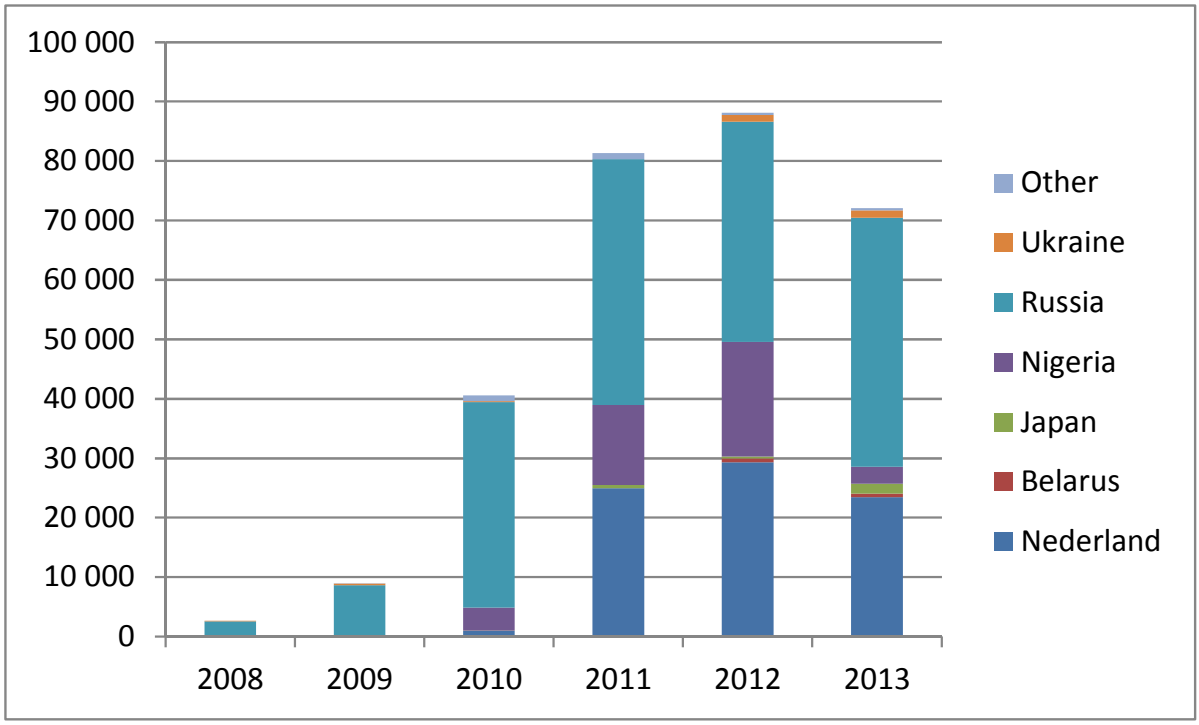



**Figure 2.7**: **Allocation of mackerel products from Icelandic processors (Hagstofa 2014)**

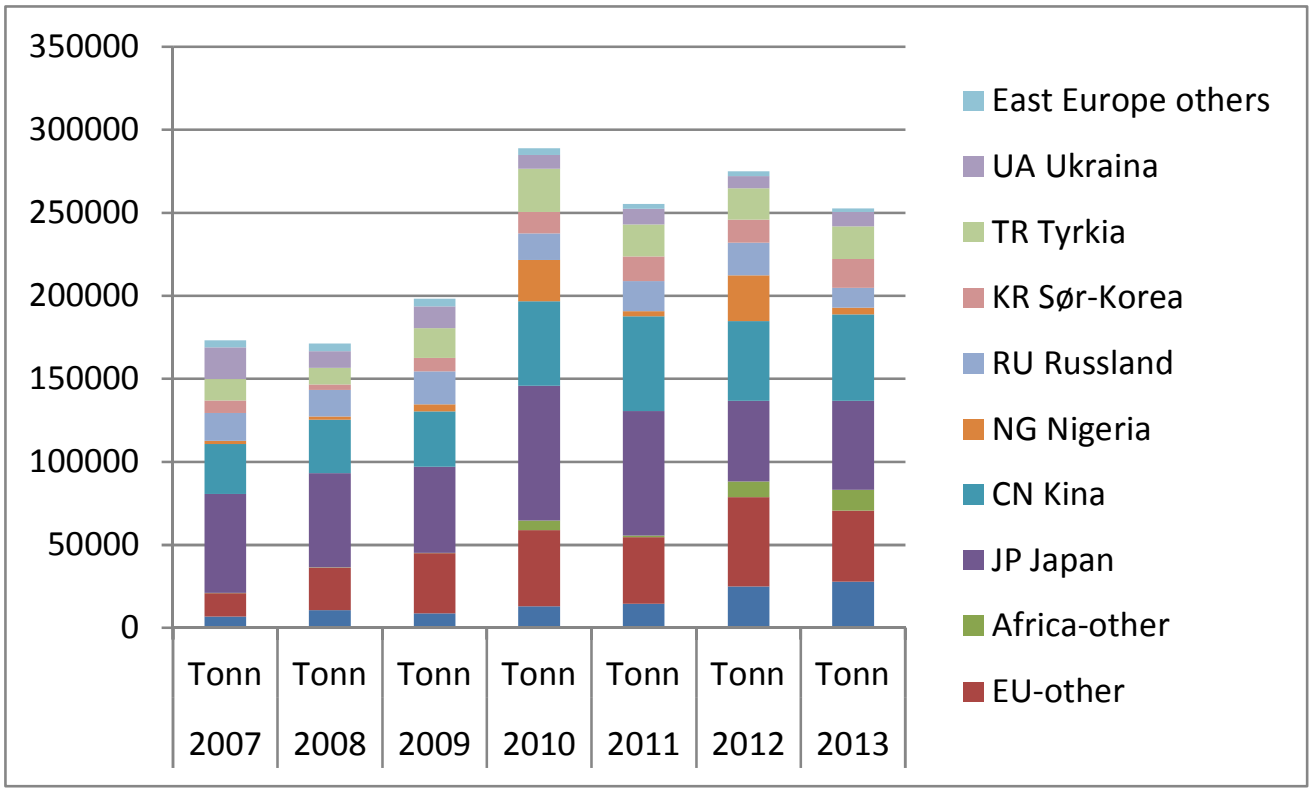



**Figure 2.8: Allocation of mackerel products from Norwegian processors (Statistisk sentralbyra 2014).**

Figure 2.7 shows that Russia is the main market for Icelandic mackerel. Only 2,4% are exported to the lucrative Japanese market in 2013. In comparison Norway exports 21% of its mackerel to Japan (Figure 2.8).

It has to be noted that these export figures do not reflect the consumption in each import nation. Mackerel exported to Netherland are often stored in Rotterdam for further export to Africa and mackerel exported to Lithuania are further processed for other markets like Germany.

Figure 2.9 shows the average price (USD) of Icelandic and Norwegian mackerel. The difference between average price (USD/kg.) of mackerel products from Iceland and Norway was 23.6% in 2009 when it was lowest and 35, 5% when it was highest in 2010. The trend from 2010 was that the difference was declining, but in 2013 it increased again.

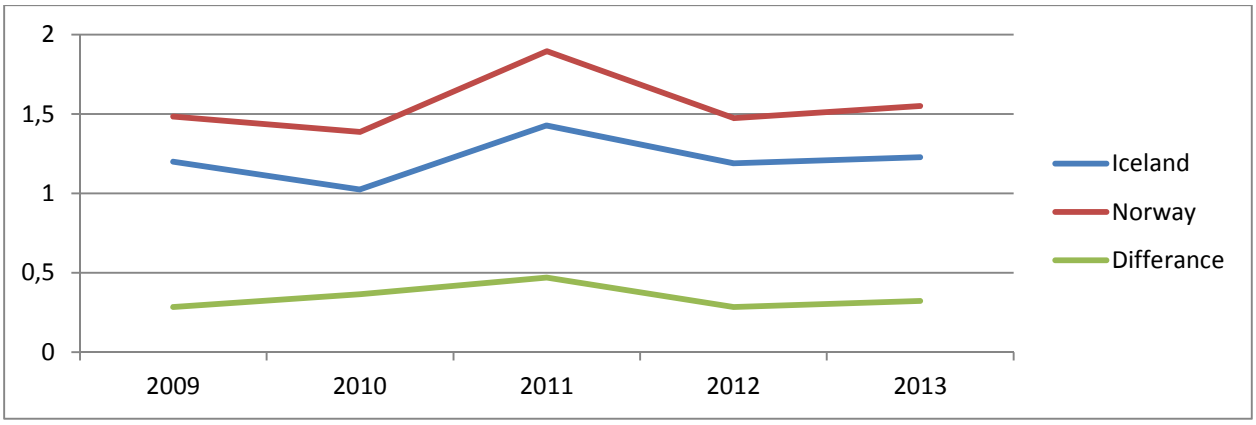



**Figure 2.9**: **Average price (USD/kg.) mackerel products from Iceland and Norway (Statistisk sentralbyra 2014, Hagstofa 2014).**

There are different product requirements and uses of Atlantic mackerel in the main markets as shown below (Gustafsson 2013, Pelagia 2014):

**Russia:** Lipid content and texture are not important for the Russian market. Price is very important. Some feed content is also tolerated. The Russians prefer whole frozen and H/G mackerel. The mackerel is used for further processing, smoking and canning. Minimum lipid content for smoking (15%), low lipid products goes to canning. UK used to be large exporter of mackerel to the Russian market, but in recent years Iceland has "taken over" mainly because of H/G mackerel suited for smoking.

**Vest Africa**: Low quality market. Prefer small whole mackerel, because it cheaper. Bad texture and some feed content accepted. Mackerel is mainly used because it is a good source of protein, rather than delicatessen. The mackerel is mainly grilled or cooked before consumption.

**Japan:** High quality market. Prefer large whole mackerel (600+) with high lipid content, firm texture, low feed, and no damages. Have to be blast frozen because contact freezing will damage the mackerel. Only mackerel caught in the winter season is accepted. Most of the mackerel is further processed in Japan, filleted and grilled for most parts, but also used for sushi.

**East Europe.** Buy H/G and whole mackerel as a raw material mainly for further processing like canning and smoking, Poland and Lithuania are the main markets for further processing. In general low fat mackerel caught early in the season is suited for canning, for smoking and marinating the lipid content has to be over 20%, but lower than 26%. Soft mackerel and some feed content are accepted.

**Turkey:** Buy whole frozen mackerel of low quality for further processing like canning.

**China:** For the most parts buy mackerel for further processing and export to Japan. The processing costs are lower in China compered to Japan. There are same quality- and raw material requirements as in Japan.

## 2.4. MACKEREL FOR HUMAN CONSUMPTION

### 2.4.1 SMOKED MACKEREL

One can divide the fish smoking process in two basic categories: cold smoking and hot smoking. In the cold smoking processing the smoking of the product takes place at a temperature up to 33 °C. In this way, one avoids the intense thermal processing and nutrients' structure is preserved. The consequence will be cold smoking does not provide adequate protection against harmful microorganisms and further processing is required prior to consumption. During cold smoking procedure the fish muscle is not cooked and no protein coagulation is taking place. Although for the majority of cold-smoked foods further thermal processing is required before consumption, smoked salmon is directly consumed (Bannerman 1980). The cold smoking involves three consecutive steps: salting, drying and smoking at temperature lower or equal to 30 °C (Montero *et. al. 2003*). Cold smoked mackerel product in Russia has 2-3 days shelf life. In most cases cold smoked mackerel are canned before consumption (Isfelag 2011).

During hot smoking temperature of the product may reach up to 70–80 °C. The above process results in cooking of the fish thus making it suitable for direct consumption (Bannerman 1980).

Typically hot smoking procedure under mild condition for whole mackerel, using frozen raw material, is shown in Figure 2.10.

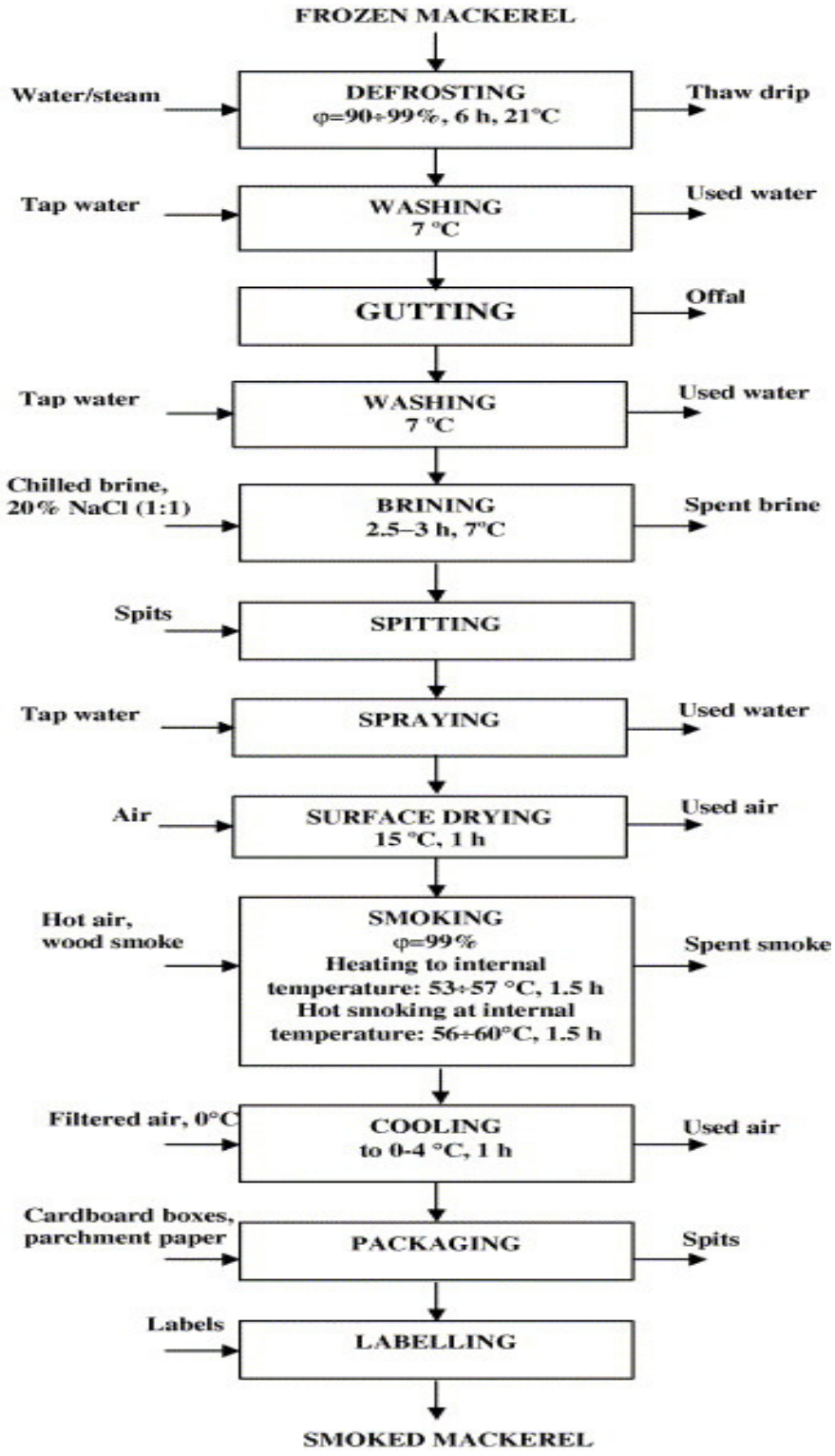



**Figure 2.10** The flow sheet of hot smoking of Atlantic mackerel in mild conditions (Stolyhwo *et. al.* 2004)

For a product of good eating quality, mackerel with a lipid content of at least 10 per cent is preferred for smoking. The smoked product can be whole gutted fish, with or without the head on, or fillets (Keay 2001).

The first step is defrosting the frozen mackerel by air or water. With 21°C running water defrosting takes about 6 hours (Stolyhwo *et. al.* 2004). Fishes processed to produce smoked products should always be fresh because the use of stale fishes can have a negative impact on final product quality (Bannerman 1980).

Second step is washing and gutting the mackerel and then wash it again after gutting. For smoked whole fish, one guts the mackerel first, or headed and gutted, as required, the gut cavity cleaned and the black belly wall lining removed. For hot smoked fillets, single fillets with skin left on are cut from the whole fish (Keay 2001).

The third step is brining. Prior to hot smoking, fishes are immersed in brine (usually 80 °) to get the necessary flavour. Higher brine concentration can also be applied for a shorter period, but it can cause spotting on fish skin due to salt crystallization (Bannerman 1980). Brining requires some care. In order to minimize the risk of food poisoning organisms growing in the finished product it is recommended that the salt concentration in the water phase of the product should be at least 3%; this concentration expressed in terms of water plus the solid components of the flesh is less than 3 %. To measure the salt concentration in the water phase it is also necessary to measure the water content of the product. A salt concentration of 3% does not render hot smoked mackerel unacceptably salty to the consumer according to Keay 2001).

According to (Keay 2001) the rate of uptake of salt during brining depends principally on the size of the fish; large fish take longer to brine than small ones. Thus to achieve a reproducible uptake of salt all the fish in a batch should be of about the same weight

A gutted mackerel of average size and fat content has to remain in the usual 80° brine for 5 hours before the salt concentration reaches the recommended 3 per cent, making it difficult to complete the whole process of gutting, brining and smoking in a normal working day. As an alternative, the use of a weaker brine for a much longer period allows the fish to lie in brine overnight. In table 2.1 is a guide following brining conditions, give a satisfactory product.

**Table 2.1: Brine strength vs. brining time (Keay 2001).**

| Weight of headed, gutted fish g | Brine strength | Brining time |
|---|---|---|
| 200 | 40° | 17 hours |
| 300 | 45° | 17 hours |
| 400 | 50° | 17 hours |

Mackerel fillets take a much shorter time to brine; an average fillet takes about 3 minutes in 80° brine.

Forth step is spitting, spraying and surface drying.

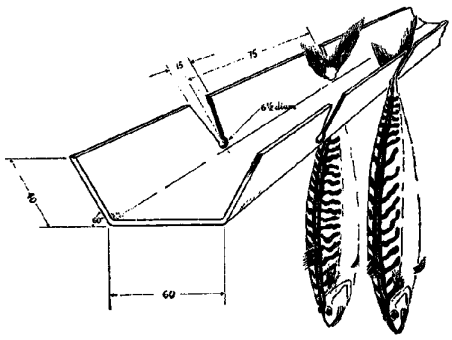



**Figure 2.11: Whole mackerel ready for smoking (Keay 2001).**

Brined whole fish can be tied by the tails in pairs and hung over tender rods for smoking, or hung individually by the tails in keyhole slots cut in metal frames designed for the purpose as shown in the drawing (Figure 2.11). For mackerel with heads on spear can be plugged through the eyes. Fillets are laid on trays made of nylon coated or stainless steel wire mesh. Trays are loaded on trolleys which when fully loaded can be left to drain for 1-2 hours or wheeled directly into the kiln without draining. In kilns with more than one trolley, the first loaded trolley should be placed at the air outlet end, and the last loaded trolley at the air inlet end (Keay 2001).

The fifth step is the smoking process. The smoking procedure for whole mackerel in a mechanical kiln is as follows. With the kiln thermostat first set at 30°C, the air inlet half to

three quarters open, and the main fan and chimney fan on, the smoke producer is made to produce maximum smoke, the recirculation damper being set to balance the smoke pressure in the kiln. This pre drying and smoking period takes ¾-1 hour, during which time surplus moisture dries off the surface of the fish, and the skins begin to set. The thermostats are then reset at 50°C, the air inlet reduced to a quarter open, and the smoke pressure balanced by resetting the recirculation damper. Temperature and humidity in the kiln begin to rise, and the fish start to cook; high humidity prevents excessive weight loss. Once the temperature reaches 50°C it stays like that for ½ hour. The skin of the fish now feels firm and colour begins to develop. For the final cooking stage, the order of the trolleys is reversed and each trolley is reversed; in a single trolley kiln, the trolley is reversed. The thermostats are reset at 80°C, the air inlet closed and the recirculation damper opened only just enough to relieve any backpressure on the smoke producer. By the time the kiln temperature reaches 80°C, small mackerel will need a further 40-45 minutes to complete the process; larger fish need about 75 minutes. As a rough guide, the whole smoking process should take about 3 hours. The fish attain a dark golden brown colour, and the skin is dry with a silky sheen. The flesh should be completely cooked; the thickest part of the flesh at the shoulders of a whole fish should be opaque, with no jellylike flakes. (Keay 2001). In the hot-smoking process, products are exposed to a wide range of temperatures (40–100 °C) while the temperature of the central point of the food can reach up to 85 °C. According to (Ünlüsayin *et. al.* 2006), the rate of deposition of different compounds depends upon the temperature, moisture, flow rate and density of the smoke, the water solubility and volatility of the particular compounds, as well as the properties of the fish surface. For example, increase in moisture and temperature can decrease the deposition rates while increase of flow rate and density of smoke causes the appearance of increased deposition rates. In the case of temperature, there is always an optimum value that has to be defined to achieve the highest deposition rates. It has also been demonstrated that many smoke substances can interact with substances in the surface of the smoked fish increasing their deposition rates.

The sixth step is cooling of the mackerel after the smoking proses. The purpose of this is to get the temperature of the product down to 0-4°C prior to packing. After hot smoking, the temperature of the product can be reduced by diverting the smoke to another point of the system and replacing it with air of decreased temperature for a period of 20 min. Another

well-applied method is the cooling of full trolleys in a low temperature room. This is vital because fish products stored without any prior cooling can be contaminated by moulds. (Bannerman 1980)

Seventh and final step is packing, labelling and storing. Several packaging materials such as cardboard, polyethylene as well as VP conditions can be used for packaging food products. One day after packaging, products obtain the desirable organoleptic characteristics. Hot-smoked foods can be effectively preserved at −30 °C for more than 6 months (Bannerman 1980).

Several factors can affect the effectiveness of the smoking method. The most important ones are raw material handling, temperature during the process, $a_w$[3], smoke characteristics and duration of smoking (Goulas and Kontominas 2005). Hot-smoking process combines satisfactorily thermal processing, which results in heating and drying, with smoking, thereby creating unfavourable conditions for the growth of harmful microorganisms and minimizing the rate of oxidative reactions (Arvanitoyannis and Kotsanopoulos 2011).

The preservation of the food is guaranteed by the antioxidant and antimicrobial properties of certain molecules (Cornu *et. a*l., 2006). For example, phenolic compounds generated by the combustion combined with the temperature and the conditions of smoking can reduce the microbiological development and the oxidation (Kjällstrand and Petersson 2001). Substances like formaldehyde and phenols are released during burning of woods imparting to smoking its preservative properties. Thanks to these substances, smoking procedure inhibits the growth of many microorganisms and limits the oxidative reactions (Muratore *et. al.* 2007). According to Varlet *et. al.* (2007), Phenolic compounds generated by the combustion combined with the temperature and the conditions of smoking can reduce the microbiological development and the oxidation. In the smoking process woods like ivory are often used for the flavouring of the food and the typical organoleptic qualities that this process confers to the smoked food.

According to US Food and Drug Administration (US FDA), product's temperature must reach 62.8 °C and should be kept under these conditions for 30 min. In this way, food pasteurization takes place thereby protecting it against harmful microorganisms (Huang *et. al.* 2002).

[3] Water activity

According to study made by Jónsson and Sveinþórsdóttir 2011, the yield from frozen raw material after brining and smoking was calculated to 84% and the composition of hot smoked Atlantic mackerel was; Water 53,4%, Protein 19,9%, Lipid 26,7% Ash 1,2%, Salt 0,18% %.

The composition of lipids in smoked products depends primarily on their contents and state in the fish used for smoking. The conditions and time of chilling and frozen storage of fish affect the rate of oxidation (Kolakowska *et. al.* 1998). Further factors influencing the state of lipids are the preparation of the raw material for smoking, the smoking itself, and storage of the products. Brining, drying, heating, and the reactivity of smoke components may have an impact on the rate of lipid changes by affecting the tissue enzymes involved in oxidation reactions, as well as by generating and changing the stability of radicals. During brining, the fish meat takes up the required quantity of salt, but also some cations present as impurities, that may have pro oxidative activity. The reaction rate of peroxidation of FA may also increase due to drying and heating in the initial stages of the process, when the concentrations of phenolic smoke antioxidants in the meat are still low. The extent of loss of moisture and the duration and temperature of heating may also be important (Kolakowska *et. al.* 2003)

Smoking is applied to produce products that preserve organoleptic characteristics at higher levels and increase the shelf life of products, thus enhancing their acceptability by consumers. The qualitative characteristics of Atlantic mackerel treated with hot-smoking procedure at 60 °C were evaluated by (Kolodziejska *et al.* 2002) the NaCl content was about 14–27 g $kg^{-1}$ of flesh. Immediately after packaging, the aerobic plate count was 0–0.48 cfu $cm^{-2}$ of skin surface and 10–240 cfu $g^{-1}$ of meat (1.9-log cycle decreased compared to raw product preserved under refrigerated conditions). At 2 °C, no changes were observed for a 3-week period. After 2 weeks of storage at 8 °C, microbial levels of the flesh were about $1.8 \times 10^2$–$1.6 \times 10^7$ $g^{-1}$. Skin microbial populations remained stable for 21 days at both 2 °C and 8 °C. During storage, colour values decreased from 4.4 to 3.5 at 2 °C and 8 °C, while rancid off-odour increased from 1.0 to 1.3 at 2 °C and from 1.0 to 2.0 at 8 °C, respectively (values presented in a five-point scale). Özogul and Balikci (2011) investigated the effects of hot smoking in combination with marinating and VP (vacuum packing), on the qualitative characteristics of mackerel (Scomber scombrus) stored at 4 °C. It was proved that sensorial properties of all samples (with or without dill leaves) were degraded during storage. After storage, samples were still of

acceptable quality, according to panellists. Total volatile basic nitrogen (TVB-N) level was estimated at 24.14 mg/100 g at the start of the experiment, and several fluctuations were observed throughout storage period. TVB level was characterized by significant ($p < 0.05$) enhancement for all samples while the level of fatty acids was enhanced from 2.46 to 7.43. Neither Escherichia coli nor S. aureus was isolated during the whole storage period, while total viable counts (TVC) were maintained at decreased levels ($<10^6$ log cfu $g^{-1}$). It was finally demonstrated that the shelf life of products was 9 months and smoking in combination with marinating and VP preserved effectively the quality of the fish. Smoking process has the advantage of enriching the fish flesh with various antimicrobial and antioxidants thereby imparting a special flavour and aroma to the product (Munasinghe *et. al.* 2003)

### 2.4.2 CANNING OF MACKEREL

Canning belongs to the most important means of fish preservation (Aitken and Connell 1979; Horner 1997). Many marine fish species produce excellent canned products, supporting an important role in the field of human nutrition and an annual export value around 2 million tons in the latest years (FAO 2013). Some marine species do not adapt to canning because the flesh disintegrates under the severe thermal processing conditions. This is the case with some lean fish, with their delicate flavour and structure, which are rendered virtually unmarketable by conventional canning processes (Aubourg 2001). Mackerel may be canned as steaks, as fillets in a variety of sauces, or as a cold smoked product (Keay 2001). Canning takes place using several steps shown in Figure 2.12.

**The raw** fish can be either frozen or fresh. Frozen mackerel is thawed up by same method as when smoking (Figure 2.10). Since most species used for canning occur in glut quantities, canneries often store the raw material before it is canned. Many of the problems with canned fish have to do with the quality of the raw material, which continuously changes during **storage prior to processing**. Two main strategies have been employed in countries where a developed technology is available, namely chilled and frozen storage.

As a result, the quality of canned fish will also depend largely on the adequacy of the methods used to hold the raw material (Aubourg 2001).

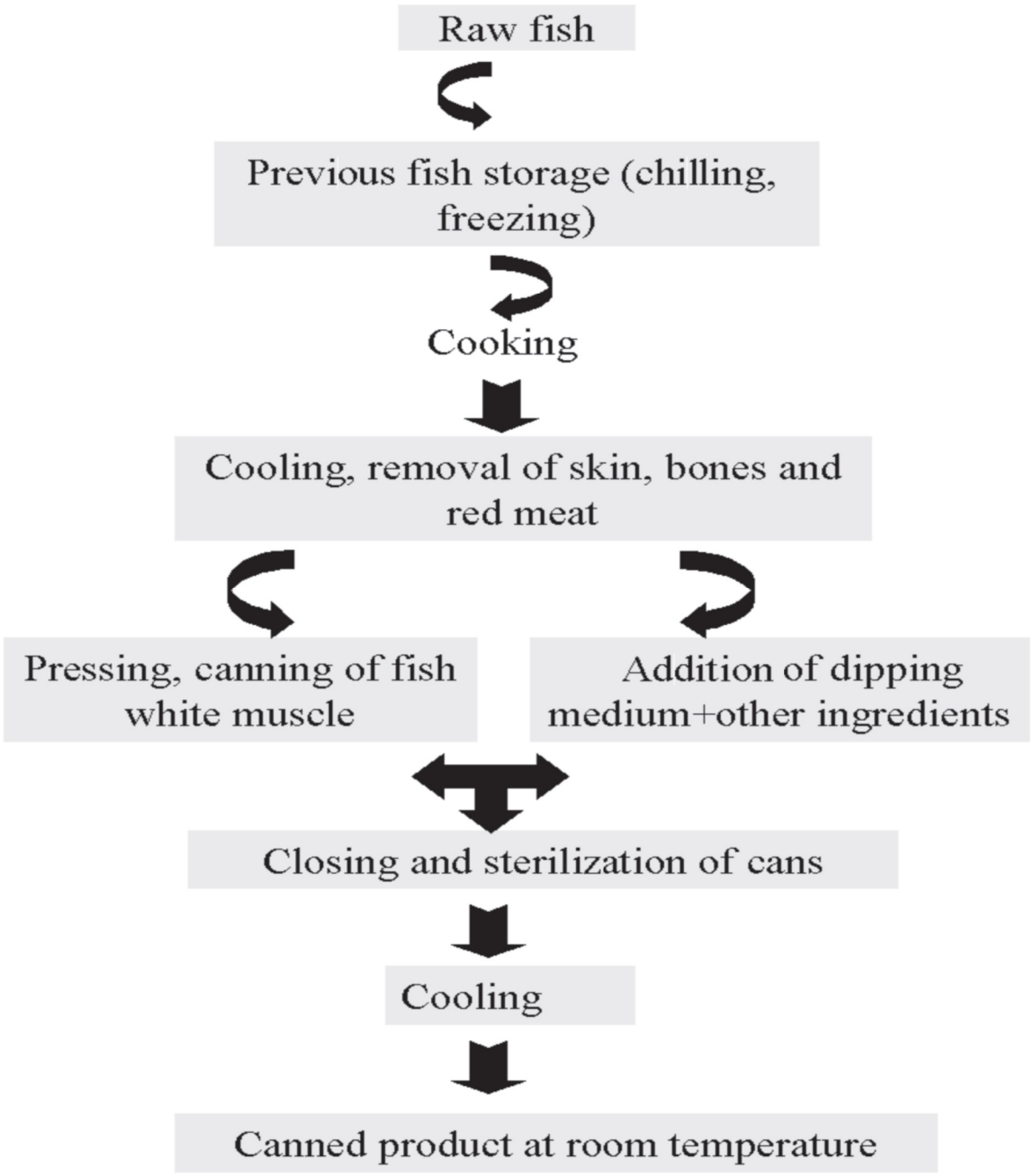



**Figure 2.12. Steps included in the canned fish manufacture (Aubourg 2001).**

The precooking method of canning cooking is accomplished using steam, oil, hot air, or smoke for 1.5 to 10 hours, depending on fish size. Precooking removes the fish oils and coagulates the protein in the fish to loosen the meat

As with any other treatment, canning should be to retain as much as possible of all the nutritional constituents present in the initial matter to serve human nutrition. The extensive

heat treatment involved in the cooking and sterilization steps substantially alters the nature of the raw material so that, in effect, a product with different characteristics is formed. Thus, both enzymes and bacteria should be permanently inactivated by heat and, provided reinfection does not occur and no negative interaction with the container is produced, heat processed fish keeps for a very long time.

Canned mackerel steaks are prepared in the following way. The whole fish is headed and gutted, and the belly cavity cleaned out. The black skin is removed from the belly wall, and traces of blood along the backbone are brushed away. The fish are then cut into steaks of a length to suit the pack; for example, steaks 6-7 cm long are suitable for a 220 g cylindrical can. The pieces are packed vertically in the can to give a product that resembles a middle cut steak of a larger fish like tuna or salmon. After adding 3 g of salt, the can is closed and heat processed for 90 minutes at 115°C. Vegetable oil, mackerel oil or tomato sauce can be added to the can before closing, or the pack can be left dry to suit particular markets. The finished cans should be stored for about 1 month before labelling and distribution; there is no further significant change in flavour or texture during storage after that time (Keay 2001).

Mackerel fillets or pieces for canning in a sauce are first soaked in saturated brine for 6 minutes, then packed into 200 g oval or ring-pull cans and steamed for 10 minutes. Any liquor in the can is then poured off, the can is filled with hot sauce, closed and heat processed for about 60 minutes at 115°C, the time depending on the ratio of fish to sauce. Variety of sauces are used; port, cider and sweet-and-sour sauces are acceptable during trials (Keay 2001). During the canning process, cooking reduces excess moisture, so that the total exudate released in the canned product is minimum, the sensory, physical and chemical qualities of the product are improved and the shelf life is prolonged. After cooking and before being placed in cans, the fish is chilled in canneries by allowing it to stand at room temperature (12–18 °C), usually overnight. The length of cooking depends on fish weight and initial temperature (Pérez-Martín *et. al.* 1989). After processing, a relative increase in lipid content occur due to water loss (Gallardo *et. al*., 1989; García Arias *et. al*., 1994). Losses in moisture increase with the previous chill time, and fish having medium fat content lose more weight and moisture than those with higher fat content (Joshi and Saralaya 1982). Damage pathways have been observed during the cooking treatment concerning the nutritional and sensory

qualities, especially if over-processing is carried out according to Cheftel and Cheftel 1976 and Pigott and Tucker 1990, they also found out that heat degradation of nutrients, oxidation of vitamins and lipids, leaching of water-soluble vitamins, minerals and proteins and toughening and drying of fragile protein fish.

### 2.4.3 MACKEREL PATE

According to Keay 2001 a delicious pate can be made from the flesh of hot smoked mackerel. The flesh is taken off the whole smoked fish, and all bones are carefully removed. For commercial production, this can be done satisfactorily in a mechanical separator. The flesh is mixed with softened or melted butter in a high-speed blender, using seven parts by weight of fish to three parts of butter. When the mixture is of smooth consistency, flavouring or spices are often added if required; for example, lemon juice and pepper can be used. Manufacturers can readily devise their own variations on the basic recipe. Some white fish flesh is sometimes added to compensate for variations in the fat content of the mackerel flesh in the mix.

The product is not sterile, and should not be kept more than 1 day at ambient temperature, or more than 5 days at 0-2°C. The pate can be frozen and cold stored in suitable containers, or it may be canned after adding a stabilizer.

### 2.4.4 SALTED MACKEREL

Quantities of mackerel were at one time pickle cured in barrels for export, and small amounts are still occasionally packed in this way.

Fresh mackerel are split along the back, and opened with a jerk to break the ribs. Guts, gills and any protruding bones are removed, and blood is washed away from the backbone. The fish are soaked in fresh water for up to 2 hours, rinsed, drained and then dipped in dry salt. The fish are packed in barrels in circular fashion with tails to the centre, and salt is sprinkled on each layer. The first two or three layers are packed skin downwards, and succeeding layers skin upwards. About 1 kg salt to 3 kg fat mackerel or 4 kg lean mackerel is required throughout the pack. When the barrel is full it is closed, laid on its side, topped up with brine through the bung and left for 10-12 days, further brine being added if required to keep the barrel full. The fish are usually graded and repacked for shipment; the fish are repacked in the barrel in the

same way, but with less salt, about 1 kg salt to 6 kg fish. The barrels are topped up with 95° brine, the bungs are closed and the barrels set on end for labelling and dispatch. Fillets of mackerel can be pickle cured in barrels in the same manner as split mackerel. (Keay 2001)

### 2.4.5 MARINATED

Marinating, a food-preservation technique, is based on treatment of muscles with solutions containing salt, spices, curds, lemon juice, etc., and provides high sensory acceptability to a variety of meat products (Yashoda *et. al.* 2005). Marinated fish is treated with acids (acetic or citric), salt, sugar, spices and oil in order to improve the flavours and textural properties of the seafood (Hwang and Tamplin 2005). Marinating process slows down the bacterial and enzyme activity and provides taste, tenderness, textural and structural changes with a prolonged shelf life (Sallam *et. al.* 2007).

I Japan mackerel fillets are marinated in different way making numerous products. One of these products is Shimesaba with annual production of 5-6000 tons. For making this product mackerel fillets are cured in 10% brine for 1 day and then soaked in marinade broth for 1 day. The broth contains different ingredients distinguished to each individual manufacturer often though containing 30% vinegar (Furutani *et. al*. 2013)

## 2.5 THE STRUCTURE AND COMPOSITION OF MACKEREL

### 2.5.1 CHEMICAL COMPOSITION OF ATLANTIC MACKEREL

As with many animal products, fish and fishery products contain water, proteins and other nitrogenous compounds, lipids, carbohydrates, minerals and vitamins. However, the chemical composition of fish varies greatly from one species and one individual fish to another depending on age, sex, environment and season (Huss 1995). What characterize pelagic fish species as Atlantic mackerel, are huge variations in compositional parameters depending on season and food availability (Hamre *et. al*. 2003). Table 2.2 shows chemical composition in mackerel caught in Icelandic waters and Norwegian waters. Huge variations are in lipid (5% - 28%) and water (53.9%-67.3%) for mackerel caught in Icelandic water compared to mackerel caught in Norway. The protein content is however more stable than the water and lipid content. According to Huss (1995), species performing long migrations, like Atlantic mackerel,

before they reach specific spawning grounds may utilize protein in addition to lipids for energy, thus depleting both the lipid and protein reserves, resulting in a general reduction of the biological condition of the fish. According to Braekkan and Boge (1962) amino acid, composition of mackerel muscle protein is similar to other common commercial fish spices, except the content of histidine where it is about twice as high (4.5%).

The chemical composition of mackerel, like most fish species, relates closely to feed intake, migratory swimming and changes in connection with spawning. The single factors having the most pronounced impact on the chemical composition is probably the feed composition (Huss 1995).

**Table 2.2:** Chemical composition of Atlantic mackerel filets per 100g/eatable part from mackerel caught in the fishing seasons in Iceland (summer) and Norway (winter) (average). (Ísgem 2011, Nifes 2006)

| | Iceland | | Norway | |
|---|---|---|---|---|
| Nutrient | 100 g/eatable part | Range | 100 g/eatable part | Range |
| Protein total | 19,6 | 18,4 – 21 | 16,9 | 15,7-17,6 |
| lipid total | 18,8 | 10,5– 24,2 | 32 | 30-34 |
| *Saturated* | 4,5 | | 10 | 12-14 |
| Carbohydrate | 0 | | 0 | |
| Water | 60,1 | 53,9-67,3 | 51 | 49-53 |
| Minerals | 1,2 | 1,1-1,6 | 0,9 | 08-1,1 |

### 2.5.2 SEASONAL VARIATIONS IN LIPID CONTENT OF ATLANTIC MACKEREL

The lipid content in fillets of Atlantic mackerel vary with season, like it does with all fatty fish (Figure 2.13), with a maximum of 25–30% around in the autumn and beginning of winter (when the fish is well fed) and a minimum of around 5% in May when the fish spawns. Those variations have great impact on the shelf life and quality of the mackerel (Wallace 1991, Huss 1995).

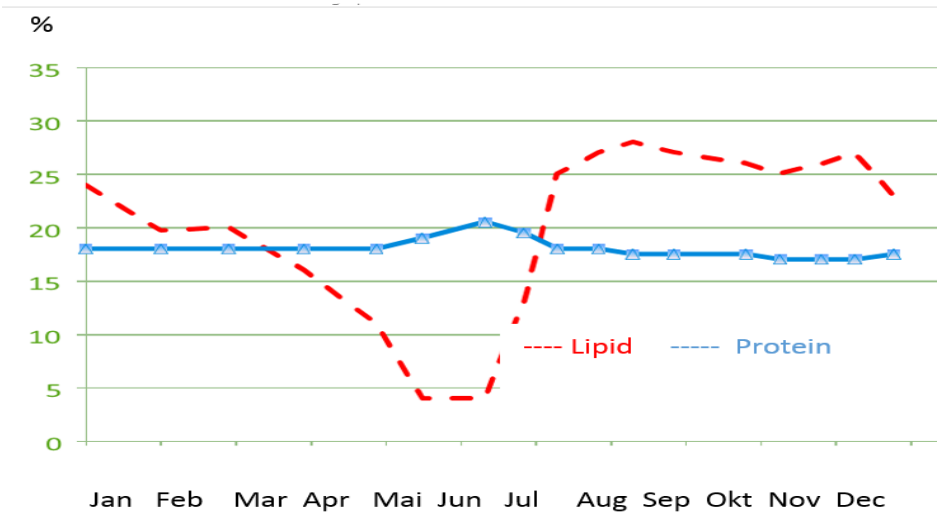



**Figure 2.13: Seasonal variation in the chemical composition mackerel fillets *(Scomber scombrus)* (Huss 1995).**

Fish will have starvation periods for natural or physiological reasons (such as migration and spawning) or because of external factors such as shortage of food. Spawning of mackerel that takes place in the spring calls for higher levels of energy. Fish having energy depots in the form of lipids will rely on this (Huss 1995).

The study of (Duinker and Pedersen 2014), taken place in Norwegian waters, showed that almost all body lipids in mackerel were missing in the spring, right after spawning. Only a small beam below the skin and some fat around the vertebrae was visible (Figure 2.14). At this stage, there was almost no lipid in the abdominal cavity. Instead, most lipid was in the carcass, with a thin layer just below the skin. After spawning the mackerel starts heavy feeding heavy feeding, resulting in a substantial increase in the fatty tissue. In September the lipid level peeks at about 30%. Also to be notice is that the lipid content of Atlantic mackerel changes with the size of the fish. Before spawning, the bigger fish has higher lipid content than smaller fish (Grégoire *et. al*. 1994).

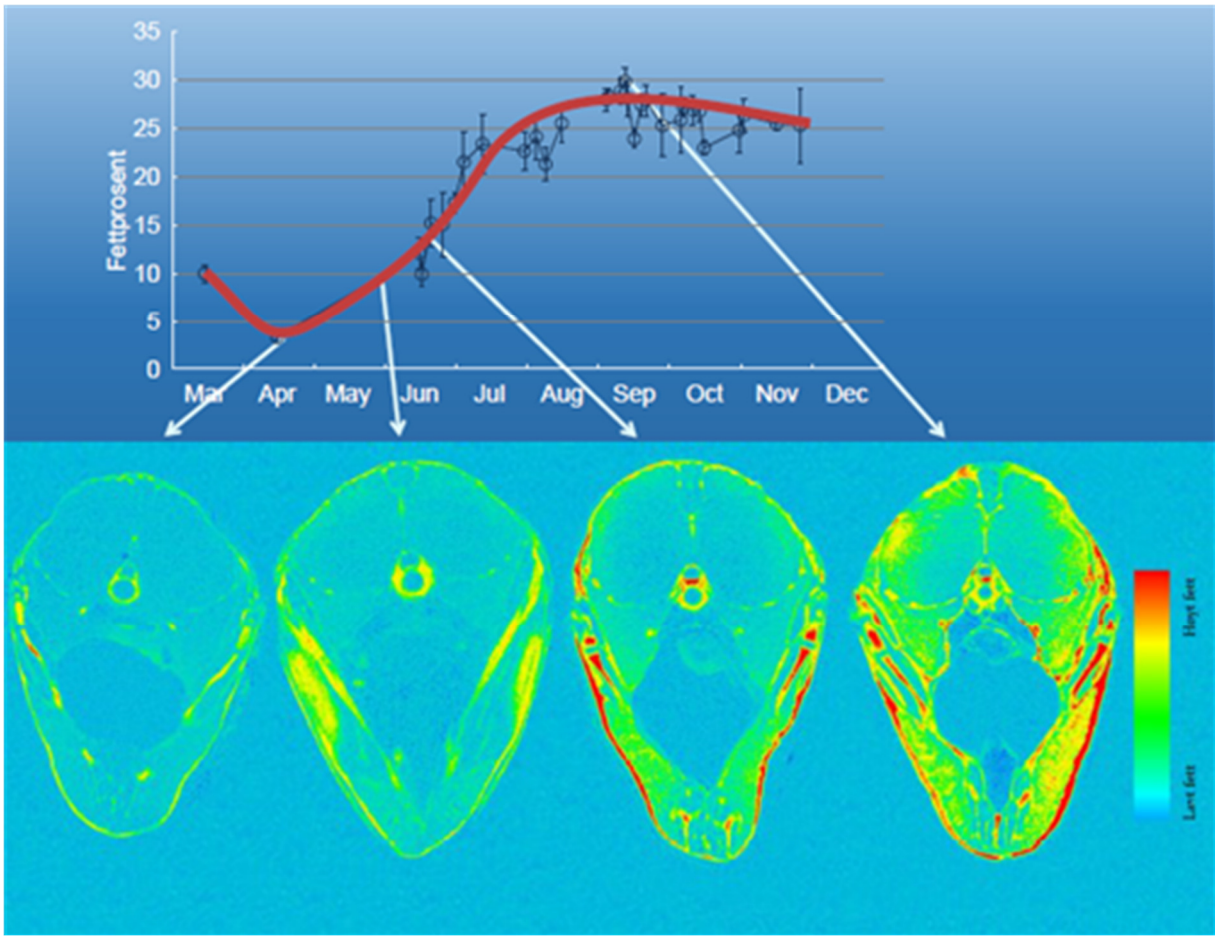



**Figure: 2.14: Seasonal variations in lipid content of mackerel (with MR imaging) (Duinker and Pedersen 2014).**

### 2.5.3 LIPID

One of the main characteristics observed in certain fish species is the ability to store up large quantities of lipids, for example, when food becomes scarce, as a source of energy for needs related to basal metabolism, migration and reproduction. In these species, it is normal to see large variations in lipid content in the course of a year. Knowledge of the annual lipid cycle of a commercial species like Atlantic mackerel is essential for fishermen and processors. Lipid content is basic factor in determine the commercial value of the Atlantic mackerel. Lipid content is considered to be one of the main factor determining further processing and allocation of the mackerel (Grégoire *et. al* 1994, MaArdle *et. al*. 1986, Pelagia 2015).

The lipid distribution in the total, intact mackerel is not well known. Furthermore, there is still a lack of understanding in the dynamics of lipid storage in mackerel. More knowledge will not only have consequences for fish nutrition and fish biology, in terms of the influence from the

prey composition on the fatty acid composition in the fish, but also for technological development used for fish localization and for the measurement of fish quantity and quality (Fjermestad *et. a*l. 2000).

There are several lipid studies on mackerel. These have mainly focused on the lipid content of the fillet and the consequences of this content on the quality of the final fish product as experienced by the consumer, and on the fatty acid oxidation in the red and the white muscle (Fjermestad *et. al.* 2000, Wallace 1991 and Gregoire *et. al.* 1994). It is clear that the maturity and fish size have effects on the lipid content of the fillet (Wallace 1991). The lipid content in the fillet inversely correlate to the growth of the gonads. Furthermore, the maximum lipid content is expected to vary from year to year (Gregoire *et. al*. 1994). It is also known that different taxonomic groups within the planktonic food web retain DHA (docosahexaenoic acid) differently, that EPA (eicosapentaenoic acid) is highly retained in zooplankton, whereas in fish DHA is highly retained (Kainz *et. al*. 2004).

The fatty acid composition in the muscle will also vary seasonally with the variation in prey consumption. Table 2.3 shows an example of fatty acid composition in mackerel caught in the North Sea 2006 (NIFES 2015). As shown here are considerable amount of polyunsaturated fatty acids chains vulnerable for oxidation.

**Table 2.3.** Fatty acid in mackerel with 32% lipid content in the North Sea 2006 (NIFES 2015).

| Fatty acid | Unit | Mean | Range |
|---|---|---|---|
| 14:0 | mg/100g | 2420 | 2197-2870 |
| 14:1n-9 | mg/100g | <1 | <1-<1 |
| 15:0 | mg/100g | 153 | 141-180 |
| 16:0 | mg/100g | 3586 | 3232-4055 |
| 16:1n-7 | mg/100g | 1056 | 931-1223 |
| 16:1n-9 | mg/100g | 57 | 46-64 |
| 16:2n-4 | mg/100g | 92 | 73-112 |
| 16:3n-3 | mg/100g | | <1-56 |
| 16:4n-3 | mg/100g | 127 | 92-154 |
| 17:0 | mg/100g | 94 | 81-116 |
| 18:0 | mg/100g | 574 | 519-652 |
| 18:1n-11 | mg/100g | 116 | 89-167 |
| 18:1n-7 | mg/100g | 454 | 387-544 |
| 18:1n-9 | mg/100g | 2425 | 1981-2898 |
| 18:2n-6 | mg/100g | 521 | 479-588 |
| 18:3n-3 | mg/100g | 578 | 525-674 |
| 18:4n-3 | mg/100g | 1908 | 1736-2403 |
| 20:0 | mg/100g | | <1-44 |
| 20:1n-11 | mg/100g | 264 | 204-330 |
| 20:1n-7 | mg/100g | | <1-42 |
| 20:1n-9 | mg/100g | 2988 | 2634-3686 |
| 20:2n-6 | mg/100g | 93 | 74-107 |
| 20:3n-3 | mg/100g | 55 | 39-64 |
| 20:3n-6 | mg/100g | | <1-44 |
| 20:4n-3 | mg/100g | 401 | 363-442 |
| 20:4n-6 | mg/100g | 133 | 112-150 |
| 20:5n (EPA) | mg/100g | 2349 | 1937-2763 |
| 22:0 | mg/100g | <1 | <1-<1 |
| 22:1n-11 | mg/100g | 4776 | 4248-6110 |
| 22:1n-9 | mg/100g | 263 | 240-300 |
| 22:5n-3 (DPA | mg/100g | 381 | 299-442 |
| 22:6n-3 (DHA) | mg/100g | 3741 | 3338-4346 |
| 24:0 | mg/100g | <1 | <1-<1 |
| 24:1n-9 | mg/100g | 442 | 391-519 |

### 2.5.4 FISH MUSLES OF ATLANTIC MACKEREL

Figure 2.15 shows fish muscle in fatty fish such as mackerel. There are two kind of muscle, dark and white. The strips of dark muscle are much larger in proportion and contain higher concentrations of lipid and certain vitamins (Huss 1995).

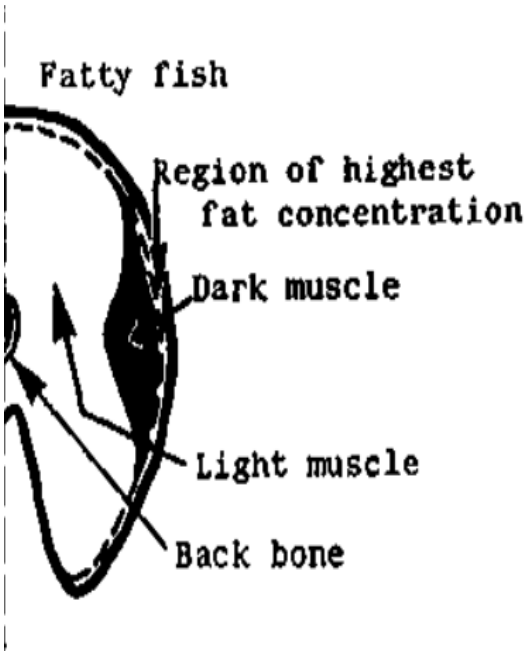



**Figure 2.15: Dark and white muscle in fatty fish (Huss 1995).**

Atlantic mackerel, like other fatty fish, store lipids in fat cells throughout the body. Figure 2.16 shows that the lipid content in the different tissues varies considerably during season.

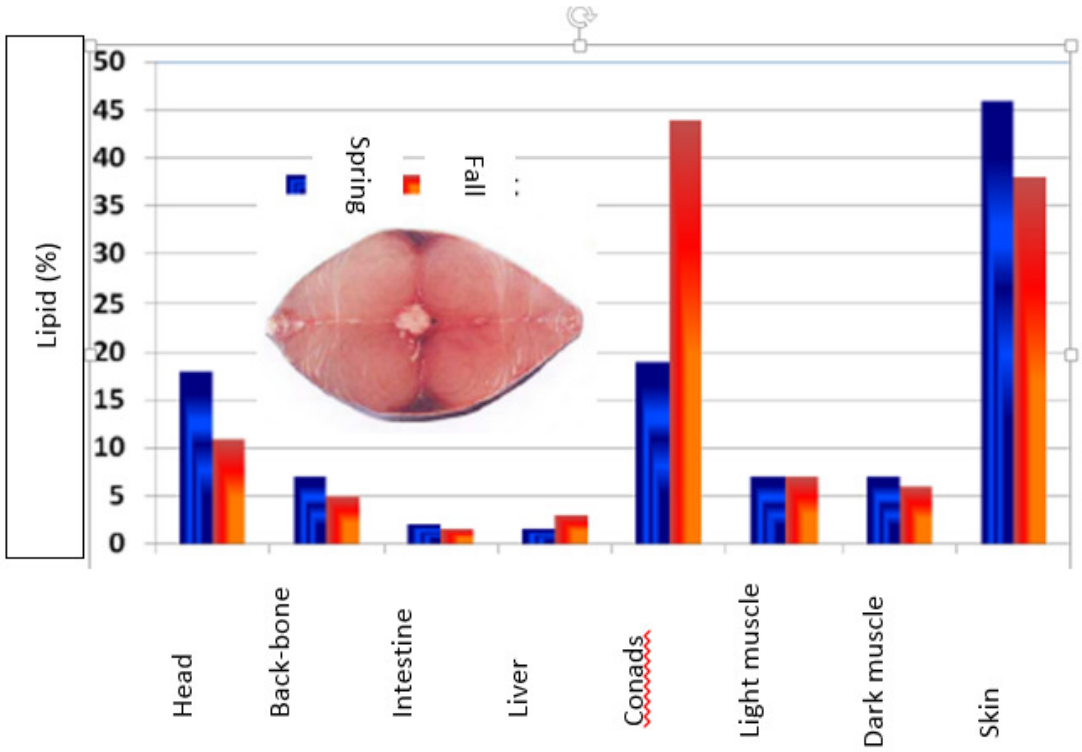



**Figure 2.16: Distribution of the total lipid various parts of the body of mackerel of Norwegian origin (Huss 1995).**

According to Huss (1995), the lipid cells making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail.

Lipid depots are also typically found spread throughout the muscle structure. The concentration of lipid cells appears to be highest close to the myocommata and in the region between the light and dark muscle (Kiessling *et al*. 1991). The dark muscle contains some triglycerides inside the muscle cells, as this muscle is able to metabolize lipids directly as energy. The corresponding light muscle cells are dependent on glycogen as a source of energy for the anaerobic metabolism.

## 2.6 SPOILAGE OF FRESH FISH

According to Hayes 1985 spoilage can be defined as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption. As soon as a fish dies, spoiling process begins. It starts with Rigor mortis. Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish mussels, when this process starts and finish is related to factors like temperature and fish species. Often it start few hours after death (Adebowale *et al.* 2008). Most fish species degrade because of digestive enzymes and lipases, microbial spoilage from surface bacteria and oxidation (AMEC 2003).

Atlantic mackerel is seldom gutted at sea; they therefore spoil quickly unless they are chilled immediately after catching and kept chilled. Furthermore fresh mackerel is more delicate for handling because the muscle is soft and fatty and the skin is not thick. According to various studies shelf life of fresh cooled mackerel is 5-7 days (Xing *et. al*. 1993) up to 9 days in ice (Jhaveri *et. al.* 1982) and 6–9 days for mackerel depending on the ice: fish ratio (Bennour *et al*. 1991). It is possible to increase the shelf life with undercooling (-2°C) and MAP or up to 21 days (Hong *et. al*. 1996).

The lipid content is also very vital. Mackerel with a lipid content of about 10% will develop off odours after 1-2 days at 10°C, will be soft and spoiling rapidly after 3 days, and will be putrid after 5-6 days (Keay 2001). Fish with high lipid content spoil even faster. Storage of fish caught during heavy feeding and or spawning seasons show wide variations in spoilage rate. Heavily feeding fish tend to be more susceptible to autolytic tissue degradation than the petite

feeders. The type of feed /food on which fish is feeding on may similarly have an effect on their spoilage rate during storage. Findings show that non-feeding fish have low levels of bacteria in the intestines as compared to the heavily feeding fish. In this sense, previous research has unveiled an important endogenous pro-oxidant activity (Huss 1995; Decker and Hultin 1990 and Saeed and Howell 2001) and quality loss during frozen storage (Jia *et. al*. 1996) and further processing (Zotos *et. al.* 1995).

Fishing technique, fishing area, handling practices, preservation, storage time, temperature, and qualitative and quantitative composition of fish micro flora also affect the quality and shelf life (Connell 1990; Burt and Hardy 1992; Gram and Huss 1996; Olafsdottir *et al*. 1997).

During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavour and texture of the fish meat. This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids (Mahmoud *et. al*. 2006). After capture, it is important to get the temperature of the mackerel down to -2°C-0°C on board the vessel. The time from catching and until the mackerel is frozen should not excide 24hours. Mackerel with a high lipid content should preferably be frozen within 12 hours from capture to prevent spoilage. Stale or noticeably spoiled mackerel is not suited for processing. The signs of stale fish are as follows: Eyes are sunken, cloudy and discoloured red or brown. The skin has lost bloom, and its colours have lost intensity and brilliance, giving a washed out appearance. Gills are dark red or brown, and a dark blood red mucus oozes from the gill covers. The odour of the gills and body is sour, sweaty or strongly oily; sometimes a smell of ammonia is also present (Keay 2001).

Fish quality (freshness) is often assessed by sensory methods based on changes in appearance, odour, colour, flavour and texture. Other analyses are also used like total volatile base nitrogen (TVB-N) who is one of the most widely used parameter to evaluate fish freshness and quality. It represents the sum of ammonia, dimethylamine (DMA), trimethylamine (TMA) and others basic nitrogenous compounds volatile under the analysis conditions. The TVB-N value is index and generally used to determine the stage of freshness of fish (along with TMA). A level of 35-40 mg N/100g of fish muscle is in general regarded as the limit of acceptability, beyond which the fish can be regarded as spoiled (Lakshmanan 2000). In freshly caught fish,

TVB-N content is generally lower than10 mg/100g and does not exceed 15 mg/100g with the exception of most pelagic fish species. Sardine, mackerel and albacore tuna have been reported to contain 16-18 mg/100g , 18-20 mg/100g about 30 mg/100 g TVB-N respectively (El Marrakci *et. al*. 1990; Malle *et. al*. 1983; Pérez-Villarreal and Pozo 1990).

### 2.6.1: AUTOLYTIC ENZYMATIC SPOILAGE

In Atlantic mackerel, the digestive enzymes are very active and begin to attack the walls of the digestive tract soon after capture. This makes the tissues even more susceptible to rupture by rough handling (Huss 1995; Stansby and Lemon 1941). Table 2.4 summarize enzymes in the digestive system of fish, their effects and prevention. The digestive enzymes cause extensive autolysis. It results in meat softening, rupture of the belly wall and drain out of the blood water, which contains both protein and oil (FAO 2005). Most of the impact is on textural quality along with the production of hypoxanthine and formaldehyde. Hansen *et. al.* (1996) stated that autolytic enzymes reduced textural quality during early stages of deterioration but did not produce the characteristic spoilage of off-odours and off-flavours. This indicates that autolytic degradation can limit shelf life and product quality even with relatively low levels of spoilage - organisms. When the belly of mackerel is full of feed the belly is very susceptible to tissue degradation and may produce the condition known as "belly burst" when the nape area of the fish takes on a brown discoloration (Bonnell 1994).

**Table 2.4: Summary of changes in chilled or frozen fish (FAO 2005 as cited by Ghaly *et. al.* 2010).**

| Enzyme(s) | Substrate | Effect | Prevention |
|---|---|---|---|
| Glycolytic enzymes | Glycogen | Lactic acid production resulting in pH drop | Avoid pre-rigor stress |
| Autolytic enzymes involved in nucleotide breakdown | ATO, ADP, AMP, IMP | Gradual production of Hypoxanthine | Avoid pre-rigor stress and improved handling. |
| Cathepsins | Proteins, peptides | Softening of tissue | Avoid rough handling during storage |
| Chymotrypsin, trypsin, carboxy-peptidases | Proteins, peptides | Belly-bursting | Problem increased with freezing/ thawing or long-term chill storage |
| Calpain | Myofibrillar proteins | Softening | Removal of calcium |
| Collagenases | Connective tissue | Softening and gaping of tissue | Time and temperature of chilled storage |
| Trimethylamine Oxide (TMAO) demethylase | TMAO | Formaldehyde | Storage temperature more than -30°C, physical abuse, freeze/thawing |

### 2.6.2 OXIDATIVE SPOILAGE

Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species with high lipid content (Fraser and Sumar 1998). Lipid oxidation in foods constitutes of a complex chain of reactions that firstly yields to primary products (peroxides), which once exposed to extended oxidation conditions, gives rise to secondary oxidation products, including

aldehydes, ketones, epoxides, hydroxyl compounds, oligomers and polymers. Most of these secondary oxidation products produce undesirable sensorial and biological effects (Marquez-Ruiz *et. al.* 2007 and Kanner 2007). Therefore, its control is a fundamental issue.

A free radical process is the basic mechanism upon which lipid oxidation proceeds. The following three stages characterize this process.

**I. Initiation: formation of fatty acid radicals**

An alkyl radical (L•) is formed due to the abstraction of hydrogen (H) from a fatty acid (L), reaction 1. Initiators (In) react by binding a hydrogen atom from an unsaturated lipid leading to formation of a free radical (McClements and Decker 2007). Reactive oxygen species (ROS) and other pro-oxidants such as transition metals, ionizing radiation or light and elevated temperature are responsible for initiation of oxidation reactions (Damodaran 2008).

**In• + LH → InH + L• (1)**

**II. Propagation: fatty acid radical reaction.**

An oxygen molecule reacts binding to the fatty acid radical leading to formation of peroxyl---fatty acid radical (LOO•, see reaction 2). Covalent bonds of unsaturated fatty acids are weak and susceptible to react with the peroxyl radicals. The reaction between unsaturated fatty acids and peroxyl radicals leads to the formation of fatty acid hydroperoxyl (LOOH, see reaction 3) and fatty acid radical (McClements and Decker 2007).

**L•+ O2→LOO• (2)**

**LOO• + LH → LOOH + L• (3)**

**III. Termination: the combination of two fatty acid radicals leads to the formation of non-radical products.**

Reactions between peroxyl and alkoxyl radicals take place under atmospheric conditions while reactions between alkyl radicals lead to the formation of fatty acid dimers under low oxygen levels (McClements and Decker 2007).

**LOO• + LOO• → LOOL + O2 (4)**

**•L• + LOO• → LOOL (5)**

**•L• + L• → LL (6)**

Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids. Fish lipids contain high amount of polyunsaturated fatty acids (PUFAs) and are therefore, are highly susceptible to oxidation. Molecular oxygen needs to be activated in order to allow oxidation to occur, but transition metals are primary activators of molecular oxygen (Hultin 1994).

The first compounds formed during the oxidation process are peroxides, especially hydroperoxides; hence, they are called primary oxidation products. Hydroperoxides usually suffer further oxidation turning into secondary oxidation products. The wide variety of secondary oxidation products includes aldehydes, ketones, epoxides, hydroxy compounds, oligomers and polymers. Among them, both volatile and non-volatile compounds can be found, such as hexanal and malondialdehyde (MDA) as main representatives, respectively.

Shahidi and Spurvey (1996) illustrated that over time there was generally an increase in thiobarbituric acid reactive substances (TBARS) value of fresh mackerel. This is attributed to the fact that during storage oxidation continues lipid hydroperoxides breakdown and produce secondary oxidation products.

Studies indicate that during food processing storage fluorescent compounds are formed as a result of reaction of primary and secondary oxidation products with biological amino compounds (Leake and Karel 1985).

Analysis of these products by using their fluorescent properties is an alternative method of assessing lipid oxidation (Aubourg 1999). Fluorescence is a rapid and sensitive method for detecting lipid oxidation. It involves the formation of covalent bonds. These bonds are strong and resistant to the action of solvents. The structure of the fluorescent compounds will vary depending on the Iipid and protein groups involved. Biological amines may react with lipid hydroperoxides and aldehydes (reactions 1 and 2). Both these reactions produce a Schiff base, which fluoresces and can be measured. The conjugated double bond system is enlarged if this reaction occurs several times (reaction 3).

**Reaction 1:** R-CHR'-OOH + R''-$NH_2$ --→R-OH + R'-CH=N-R"

(lipid hydroperoxide) (primary amine) (Schiff base)

**Reaction 2:** R-CHO + R'-$NH_2$ --->R-CHOH-NHR' → R-CH=N-R'

(aldehyde) (primary amine) (Schiff base)

**Reaction 3:** R-CH2-CH=N-R' + R''-CHO → R-C=CH-R"

(Schiff base) (aldehyde) I

CH=N-R'

(conjugated Schiff base)

### 2.6.3 LIPID HYDROLYSIS

Progressive lipid hydrolysis is shown as free fatty acids FFA. FFAs are either important from the point of view of oxidation products or have been reported to have a direct sensory impact. In figure 2.17 lipid hydrolysis were FFA are formed is shown. Triglyceride in the depot fat is cleaved by enzyme (triglyceride lipase) originating from the digestive tract (HUSS 1995).

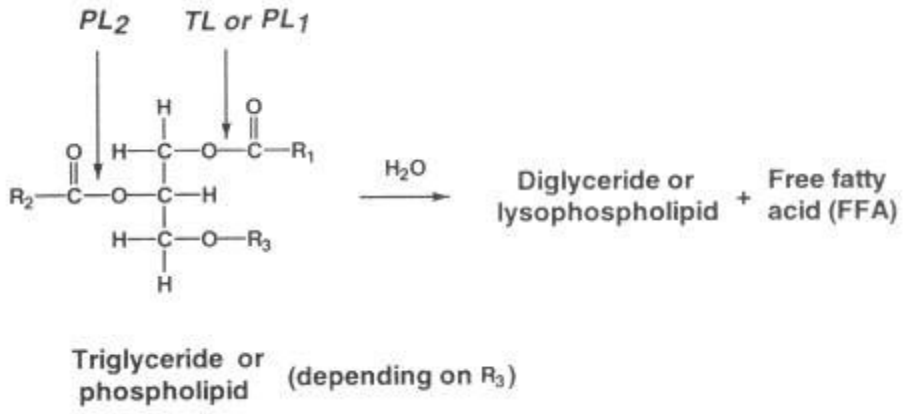



**Figure 2.17**. Primary hydrolytic reactions of triglycerides and phospholipids. Enzymes: $PL_1$ & $PL_2$ phospholipases;TL, triglyceride lipase (Huss 1995)

In Figure 2.18, examples of rate of oxidation and breakdown of lipid are shown in relation with time and different analysis method.

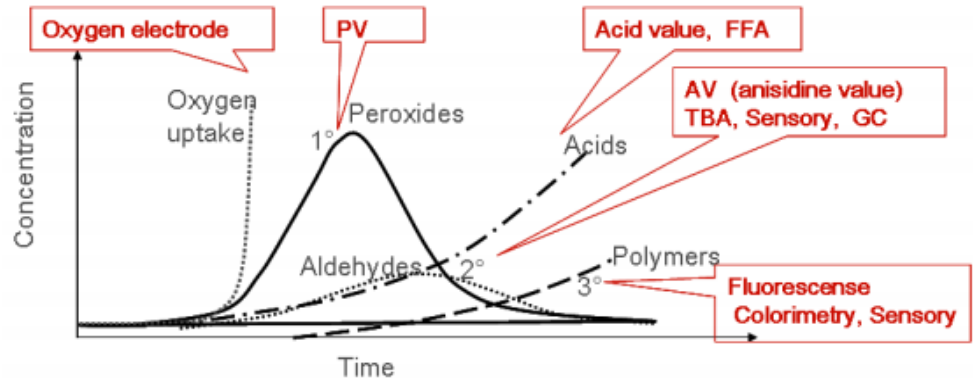



**Figure 2.18: Example of oxidation and breakdown of lipid and different analyses methods. 1° oxidation is analysed as PV (peroxide value). 2° oxidation is measured by TBA. 3° oxidation can be measured by fluorescence (Jonsdottir *et. al*. 2008).**

## 2.7 EFFECTS OF FREEZING AND STORAGE ON ATLANTIC MACKEREL

Freezing and frozen storage of fish have largely been employed to retain sensory quality and nutrients (Erickson 1997). When fresh raw material is properly frozen and then kept at a sufficiently low temperature, spoilage can be almost entirely stopped (FAO 2001). During the freezing process, free water transforms into ice crystals, which cause mechanical damage to the muscle cell structure. Frozen storage gives rise to mechanical damage through ice crystal formation (Bello *et. al.* 1982; Ayala *et. al*. 2005) and protein denaturation (Fennema 1990) which can have impact on final product quality (Sigurgisladottir *et. al.* 2000).

Lipid oxidation is the main reason for quality deterioration in frozen stored fatty fish species such as mackerel (Shenouda 1980; Hultin 1994) due to high content of polyunsaturated fatty acids (PUFAs). PUFAs are highly susceptible to lipid oxidation during frozen storage, which results in rancid taste and odour, especially if the fish products are not packed in an oxygen-free atmosphere (Nielsen and Jessen 2007). Even though frozen storage slows down quality degradation, some quality decline of mackerel occurs at extra-cold storage (Chapman *et. al*. 1993). With increased time, the quality of mackerel decreased according to sensory and chemical indices but the extra-cold storage temperatures however slowed the deterioration as measured by rate of hardening of mince and slower free fatty acid (FFA) formation.

Compositional changes during fish spoilage result in lipid oxidation and protein degradation as well as the loss of other valuable molecules. In order to develop optimum preservation

techniques for these compounds, understanding of the mechanism responsible for their degradation is essential. Some deterioration of the frozen product takes place during frozen storage (Figure 2.19), but the changes are so small under the right conditions that to the ordinary consumer the thawed product is indistinguishable from fresh after many months in store. The lower the holding temperature, the longer is the period of safekeeping; hence, temperature of storage is the most important single factor affecting the storage life of frozen fish (FAO 2001).

Various other factors affect the quality of fish products after freezing and frozen storage. Factors like fish species, temperature when caught, handling on-board vessel, the biological status of the fish, temperature of the pre-rigor storage, freezing rate, frozen storage temperature and time, temperature fluctuations, thawing procedure and prevention against oxidation (light and oxygen) (Sørensen *et. al*. 1995; Sigholt *et. al*. 1997; Erikson *et. al*. 1997; Kristoffersen *et. al*. 2006; Nielsen and Jessen 2007).

The frozen storage of Atlantic mackerel (Scomber scombrus) is limited by lipid damage causing sensory quality losses, an important drawback to its commercialisation.

During freezing, damage pathways are inhibited, undesirable reactions associated with lipids and proteins have shown to occur, leading to detrimental changes in nutritional and sensory properties (Sikorski and Kolakowska 1994; Erickson 1997). Ke *et. al.* 1977 showed kinetic variations in lipid oxidation in various parts of mackerel stored at −15, −30 and −40°C. By measuring the changes of POV and of TBA molar values as a function of time. Oxidation developed in the skin sample (subcutaneous fat) was eight times faster in TBA change than in the white and dark muscles from mackerel held at −15°C for two months. This rapid development of rancidity in the skin inhibited effectively by lowering the frozen storage temperature to −40°C, where relatively the degree of lipid oxidation in the dark muscle was of the same order of magnitude. The in vitro rate of autoxidation at -60°C for the lipids extracted from skin and the fillet muscle showed an unusual difference, which indicated that unknown pro−oxidative substances in mackerel skin were fat solvent extractable. The activity of the unknown compounds in the skin was also temperature dependent even in the frozen state, but could be mitigated effectively by lowering temperature to −40°C. Lipid hydrolysis in mackerel was also retarded completely when the fish were stored at −40° C.

The slight variation in fatty acid between mackerel skin and meat samples did not seem adequate to explain the rapid oxidation in skin lipids. Thus it is believed that the skin lipids

one or more fat-solvent-extractable prooxidants, alone or associated with trace metals, were present and were responsible for the high susceptibility to oxidation found for this lipid.

According to Nair *et. al.* 1987, mackerel could be preserved safely under frozen storage (–23 °C) for a period of 23–24 weeks. Measurement of sensory, chemical and physical changes have shown that deterioration of fish quality continues to some extent during frozen storage, since undesirable changes associated with lipids and proteins occur. Studies on frozen fish have shown four different pathways of damage which lead to nutritional and sensory losses (Shenouda 1980; Haard 1992; Mackie 1993; Xiong 1997; Erickson 1997). The muscle becomes harder, more fibrous, and less elastic and loses its water holding capacity.

Toughening texture develops in edible muscle. As a result, proteins become more prone to damage and essential amino acids are more susceptible to loss. Endogenous enzymes (lipases, phospholipases; lipoxygenases, peroxidases) are still active during the frozen storage, especially if light or other catalysts (heme groups, transition metals) are present. As a result, loss of essential fatty acids are produced and a wide range of lipid oxidation and hydrolysis compounds are produced, most of them relatively small molecules, that are susceptible to interaction with fish constituents leading to a lowering in nutritional and sensory values of the product. An endogenous enzyme (TMAO dimethylase) catalyses this degradation. DMA produces detrimental odour and flavour and FA facilitates protein crosslinking, so that protein denaturation resulting in toughening of texture. Unaltered myoglobin reportedly are highly responsible for the bright of white muscle in good quality fish. During frozen storage, this protein oxidizes to metmyoglobin, leading to important sensory quality losses of the fish product by means of a browning development in muscle (Aubourg 2001).

Frozen (–25 °C) stored mackerel also showed a decrease in gel-forming capacity and jelly strength (Nishimoto and Koreeda 1979).

Jian *et. al.* 1987 studied the effect of free amino acids on denaturation of mackerel proteins during frozen (–20 °C) storage. Their conclusion was that the NH3 group of free amino acids (histidine, lysine, taurine, glycine, proline and glutamic acid) interacted with carbonyl groups on protein, so that in the case of histidine, lysine and taurine protein denaturation increased.

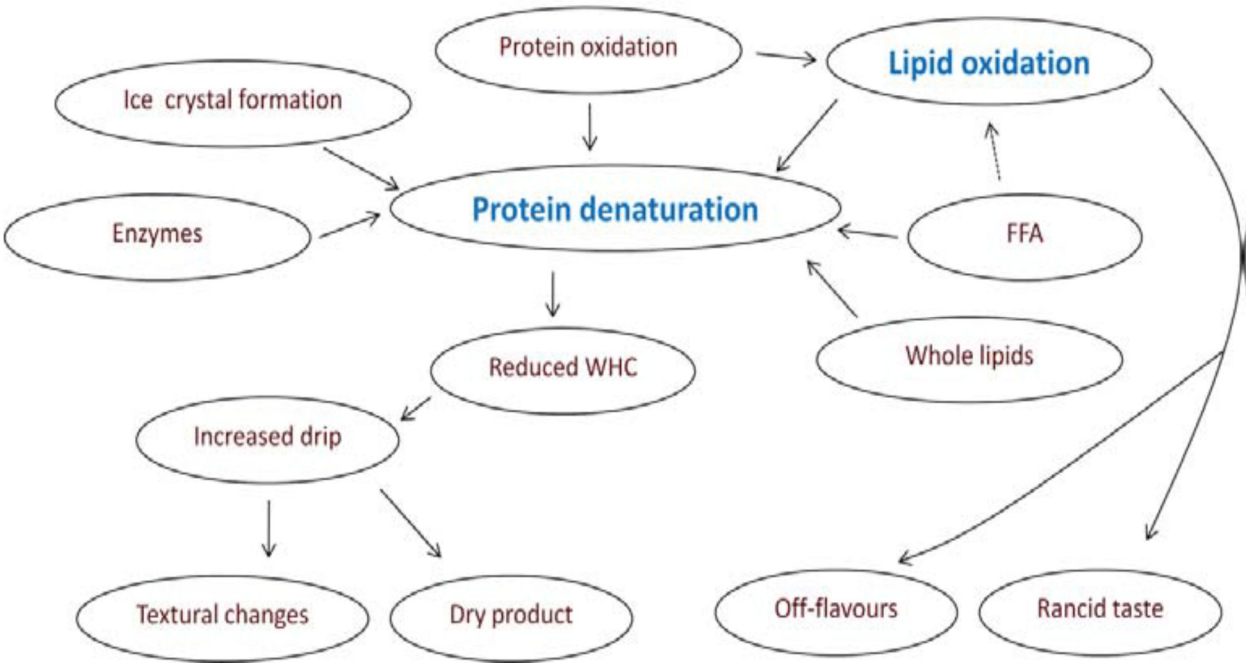



**Figure 2.19: Quality-related changes in fish muscle during frozen storage (Burgaard 2010).**

## 2.8 STUDY OBJECTIVES

Icelandic mackerel processors are constantly trying to increase the value of the mackerel products. Hindering spoilage throughout the process from catching to frozen storage is very important in order to achieve that goal. Spoilage during frozen storage is one of the factors of great interest.

The objectives of this study was to investigate the effects of storage time and temperature as well as raw material quality on the extent of lipid oxidation in H/G mackerel stored at approximate -27 °C for one and four months.

# 3. MATERIALS AND METHODS

## 3.1 EXPERIMENTAL DESIGN

The study took place during the mackerel-fishing season in Iceland from late June to beginning of September 2012. Figure 3.1 describes the experimental design of the study. Samples for evaluation of the stability of the raw material and quality of final product were taken according to common practice in Isfelag´s processing plant (Appendix B and Appendix C). Information on newly caught mackerel came from the fishing vessel Heimaey VE-1, inspection of quality of raw material after grading were conducted at the grading station and inspection on final product took place at Isfelag's processing plant.

The chemical composition and TVB-N content of fresh raw material were analysed in Rannsóknarþjónusta Vestmannaeyja in Vestmannaeyjar. For frozen samples, lipid oxidation (peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and fluorescence shift ratio (δF)) and lipid hydrolyses (free fatty acid (FFA)) were measured at Matís ohf, Reykjavik.

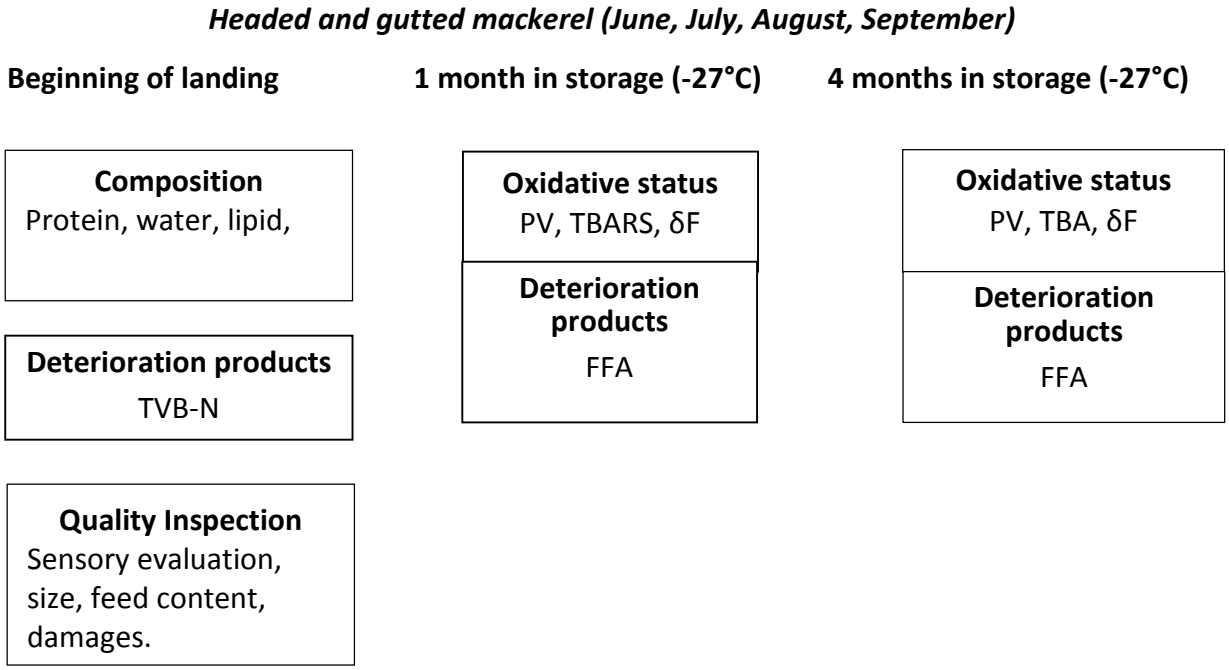



**Figure 3.1: Experimental design of the study. (Abbreviations: FFA = free fatty acids, PV = peroxide value, TBARS= thiobarbituric acid value, δF= Fluorescence shift ratio measurements. TVB-N = total volatile nitrogen).**

## 3.2 RAW FISH AND SAMPLING

The fish were caught by Isfelag´s own trawler Heimaey VE-1, in different fishing grounds at different time (Appendix A), and landed directly at grading station. A total number of five samples were collected during the fishing season from end of June to beginning of September (Table 3.1).

**Table 3.1: Dates of samples collection.**

| Sample | Day of landing |
|---|---|
| Late June | 28.06.12 |
| Late July | 25.07.12 |
| Middle August | 13.08.12 |
| Late August | 29.08.12 |
| Beginning September | 07.09.12 |

Upon arrival at the production site, ten fishes of each sample were hand filleted with skin on, wrapped in plastic foil and put into cold storage (-27 °C) for 1-2 days before taken to the laboratory[4] to be analysed for proximate composition (protein-, water- and, -lipid content) and TVN-B.

For each frozen samples, four blocks (ca. 12 kg.) of H/G mackerel, processed according to the process described in Figure 2.4, were stored in cold storage at approximately -27 °C (Appendix D). After 1 month and 4 months of frozen storage 1 block was, send to Matis laboratory to be analysed for lipid oxidation products and lipid hydrolysis. In total ten blocks of H/G, mackerel were sent to Matis. The temperature of the cold storage was monitored and reported (Appendix D).

[4] Local lab in Vestmannaeyjar (Rannsóknarþjónusta Vestmannaeyja) was used for initial proximate analyses.

## 3.3. PROXIMATE ANALYSIS

### 3.3.1 WATER CONTENT

Water content was determined by drying the samples (ISO 1983). The mackerel fillets was mixed well and about 5 g of the grounded samples weighed accurately into a pre weighted porcelain bowl, containing sea sand and a glass rod. The glass rod was used to mix the sample and the sand in order to increase the surface prior to drying. Then the sample was dried at 100°C for 4 hours. After drying the porcelain bowl was closed with the lid and the sample allowed cooling in a desiccator, prior to weighing. The water content corresponds to the weight loss and was given as percentage of the initial sample weight.

### 3.3.2. LIPID CONTENT

Lipid content was determined according to the Soxhlet method, ISO 6492:1999 (ISO 1999).

### 3.3.3. PROTEIN CONTENT

The protein content was determined by using the Kjeldahl method ISO 5983-2:2005 (ISO 2005).

## 3.4 OXIDATIVE STATUS

### 3.4.1 LIPID HYDROPEROXIDE VALUES (PV)

Lipid hydroperoxides was determined with a modified version of the ferric thiocyanate method (Santha and 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 N) was added (5.0 mL) in to the mixture and homogenized for 5 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 mixture (1:1) of ammonium thiocyanate (4M) and ferrous chloride (80 mM) was added and vortexed. The samples were incubated at room temperature for 10 min and red at 500 nm (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as µmol lipid hydroperoxides per g of samples.

### 3.4.2 THIOBARBITURIC ACID REACTIVE SUBSTANCE (TBARS)

A modified method of Lemon (1975) was used for measuring 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% ethylenediaminetetraacetic acid mixture prepared in ultra-pure water) using a homogenizer at maximum speed for 5 seconds (Ultra-Turrax T-10 basic, IKA, Germany) and centrifuged at 5100 rpm for 20 min (TJ-25 Centrifuge, Beckmann Coulter, USA). 0.1 mL supernatant was collected and mixed with 0.9 mL 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 TEP. The results were expressed as µmol of malomaldehyde diethylacetal per g of samples.

### 3.4.3 FLUORESCENCE SHIFT RATIO (δF)

Fluorescence measurements (Perkin Elmer LS 50B) were made at 393/463 and 327/415 nm excitation/emission maxima, according to other researchers (Aubourg et al. 1997; Aubourg et al. 1998; Aubourg 1999a; Aubourg 1999b; Aubourg 2001). The excitation and emission slit was set at 2.5 nm. 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 $H_2SO_4$) at the corresponding wavelength. The fluorescence shift ($\delta F$) was calculated as the ratio between the two RF values, i.e. $\delta F = RF_{393/463nm} / RF_{327/415nm}$, and was analysed on the organic phase resulting from the lipid extraction (Bligh and Dyer 1959).

## 3.5 LIPID HYDROLYSIS (FFA)

Free fatty acids (FFA) were determined on the TL extract resulting from the Bligh and Dyer (1959) extraction. Measurements were conducted according to Lowry and Tinsley (1976), with modifications from Bernardez *et. al.* (2005). The FFA concentration was calculated as micromolar oleic acid based on standard curve spanning a 2-22 µmol range. Results were expressed as grams FFA per 100 g of lipids.

## 3.6 TOTAL VOLATILE NITROGEN BASES (TVB-N)

The method of Malle and Tao (1987) was used for total volatile bases (TVB-N) and trimethylamine (TMA) measurements. 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 solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. The results were expressed as mg TVB-N/100 g muscle.

## 3.7 STATISTICAL ANALYSIS

Statistical analyses were performed by Microsoft Office Excel 2010 (Microsoft Inc., Redmond, Wash., U.S.A.).

# 4 RESULTS

## 4.1 FRESHLY CAUGHT MACKEREL

### 4.1.1 RAW MATERIAL CONDITION

Table 4.1 shows information obtained from the fishing vessel related to the raw material and the mackerel condition when caught. During the season, from end of June to middle of September, the sea temperature changed from 12.5°C in June were it peaked in July (13.5°C) and went down to 8°C in August. The age of the raw material varied from 25 hours (end of September) to 53 hours (beginning of September). Haul time increased also from about 4 hours at the beginning to about 6 hours late in the season. The temperature of the mackerel when landed ranged from -0.5 °C to - 0.8 °C for four samples. The last sample was significant higher +0.9°C compared to other samples. In that case, something had probably gone wrong in the cooling on-board.

**Table 4.1: Size group, weight of sample, tank, temperature when landed, sea temperature, age of raw material, haul time, fishing ground and average size.**

| | Samples | | | | |
|---|---|---|---|---|---|
| | **Late June** | **Late July** | **Middle August** | **Late August** | **Beg. Sept.** |
| Raw material size group (size) | 200-400 | 250+ | 250+ | 250+ | 250+ |
| Sample (kg) | 6.4 | 7.5 | 8.1 | 7.8 | 9.1 |
| Tank | 2 starboard | 2 middle | 2 middle | 1 starboard | 2 port |
| Mackerel (°C) | -0.3 | -0.8 | -0.6 | -0.8 | +0.9 |
| Sea temperature (°C) | 12 | 13 | 8 | 8 | 7.5 |
| Age (hours) | 25 | 36 | 44 | 50 | 53 |
| Haul time minutes | 250 | 240 | 270 | 300 | 360 |
| Fishing ground[5] | 272 | 322 | 462 | 462 | 512 |
| Average size (kg) | 0.32 | 0.36 | 0.40 | 0.47 | 0.50 |

[5] Appendix A

The fish was caught in three different fishing grounds as seen in Appendix A. The first two South West of Iceland and the rest east of Iceland.
Average size increased during the season from 0.32 kg. at the beginning to 0.5 kg. at the end of the season.

Quality properties of the raw material samples are listed in Table 4.2. Soft tissue was visible at the beginning of season were over 24% of the mackerel was soft and in the middle of August when 10% softness was observed, other samples had no fish with soft tissue. The feed content fluctuated from 0.2 to 1.1[6], which is according to most Product Specifications low feed content. Skin damages were visible only at the first sample. Some bloodspots were observed in samples from late June and middle of August. Some feed damages 1 were detected in sample caught late June, middle of August and early September. Feed damages 2 were only visible in samples from late June or 16.6%.

**Table 4.2: Quality of the raw material when landed at grading station (average values from several samples).**

| | **Catching season** | | | | |
|---|---|---|---|---|---|
| | **Late June** | **Late July** | **Middle August** | **Late August** | **Beginning September** |
| Firm tissue (%) | 75.9 | 96.9 | 93.5 | 100 | 100 |
| Soft tissue (%) | 24.1 | 0 | 9.7 | 0 | 0 |
| Feed content | 0.9 | 0.5 | 0.2 | 1.1 | 1,0 |
| Skin damages (%) | 11.7 | 0 | 0 | 0 | 0 |
| Feed damages 1 (%) | 40 | 0 | 57.7 | 0 | 21.6 |
| Feed damages 2 (%) | 16.6 | 0 | 0 | 0 | 0 |
| One blood spot (%) | 8.3 | 0 | 2.2 | 0 | 0 |
| Over one blood spots (%) | 4.4 | 0 | 0 | 0 | 0 |
| Damages spine (%) | 0 | 0 | 0 | 0 | 0 |

These results indicates no obvious damages due to rough handling of the raw material on board the vessel.4.1.2 Condition of the final product

[6] In Appendix B calculation of feed content is shown.

### 4.1.2 CONDITION OF THE FINAL PRODUCT

Quality parameters of the mackerel after it was processed (headed and gutted) and before it was packed and frozen are listed in Table 4.3. The fish temperature was similar for all samples ranging from 1.4°C to 1.9°C, indicating that cooling was adequate during the production process. Soft texture was measured in sample from late August 6.5 %. The other samples had soft tissue under 1.9%. Average size was similar for the last four samples 0.3 – 0.34 kg., but in the first sample average size was considerable lower or 0.23 kg. Some damages and fish with gut were observed in all the samples but middle of August were no bad gut removal was observed and only 0.25 % damages.

Quality ratio is a grade given to each lot number. If there is no remarks the quality ratio is 100%. Every remarks deducts the grade. Quality ratio of four of the samples were (95.96 5 to 99.75%) which is good. Late August did only get quality ratio of 91.19%, mainly because of 6.5% fish with soft texture.

**Table 4.3: Quality parameters of H/G mackerel before freezing (average values from several samples).**

| | **Catching season** | | | | |
|---|---|---|---|---|---|
| | **Late June** | **Late July** | **Middle August** | **Late August** | **Beginning September** |
| H/G mackerel | 200-400 | 250+ | 300+ | 250+ | 250+ |
| Temperature °C | 1.6 | 1.9 | 1.8 | 1.6 | 1.4 |
| Average size (kg) | 0.23 | 0.32 | 0.34 | 0.3 | 0.34 |
| Soft texture % | 1.43 | 1.88 | 0 | 6.5 | 1.2 |
| Firm texture % | 98.57 | 98.2 | 100 | 93.5 | 100 |
| Cutting defects % | 0 | 0 | 0 | 0 | 0 |
| Damaged % | 1.14 | 0.61 | 0.25 | 1.5 | 0.25 |
| Bad gut removal % | 1.47 | 0.29 | 0 | 0.81 | 0.87 |
| Quality ratio | 95.96 | 97.22 | 99.75 | 91.19 | 97.68 |

### 4.1.2 CHEMICAL COMPOSITION OF MACKEREL

The chemical composition of the mackerel fillets varied considerably from late June until middle of September (Figure 4.1). The lipid content increased from 15.1% in late June and peaked at 32.9% in late July, and at the end of the season, it was 25.2%. Water content was 65.3% in late June, had its lowest level in late July 51.5%. The correlation between water and lipids was measured -0.9982. Protein value changed from 15.6% at the lowest in middle of July and up to 19.1% in late August. Results from other studies shows similar results (Arason *et. al.* 2010).

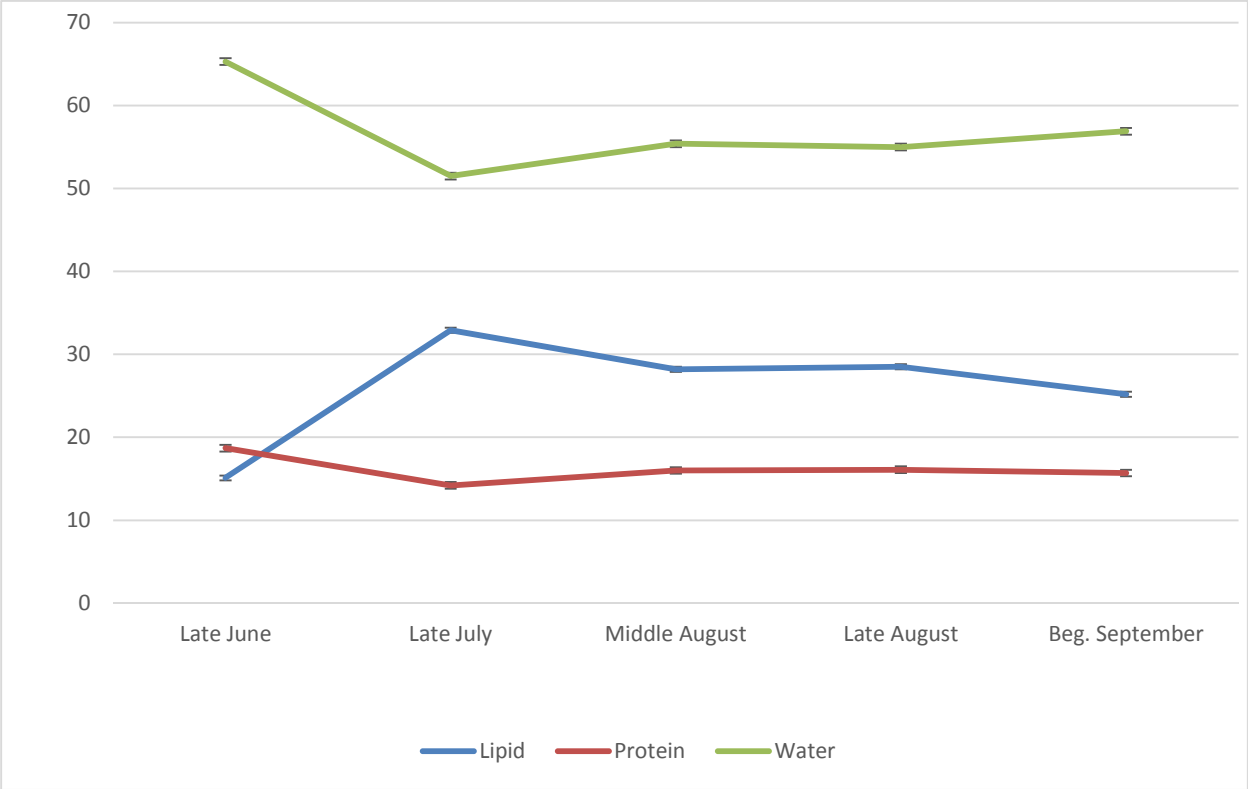



**Figure 4.1: Influence of catching time of Atantic mackerel caught in Icelandic water from June-September 2012**

### 4.1.3 TVB-N

TVB-N values of fresh mackerel varied from 15.8 to 19.1 TVB/100g (Table 4.4). The highest value was observed in late August and the lowest in middle of August.

**Table 4.4: Total volatile basic nitrogen (TVB-N/100g) for fresh mackerel**

| Sample | Fresh | STD |
|---|---|---|
| Late June | 17.7 | +/- 0.5 |
| Late July | 16.0 | +/- 0.5 |
| Middle August | 15.8 | +/- 0.5 |
| Late August | 19.1 | +/-0.5 |
| Beg. September | 17.9 | +/- 0.5 |
| **Average** | **17.3** | |

## 4.2 LIPID OXIDATION

### 4.2.1 PEROXIDE VALUE (PV)

Peroxide values after 1 month and 4 months of frozen storage is shown in Figure 4.2.

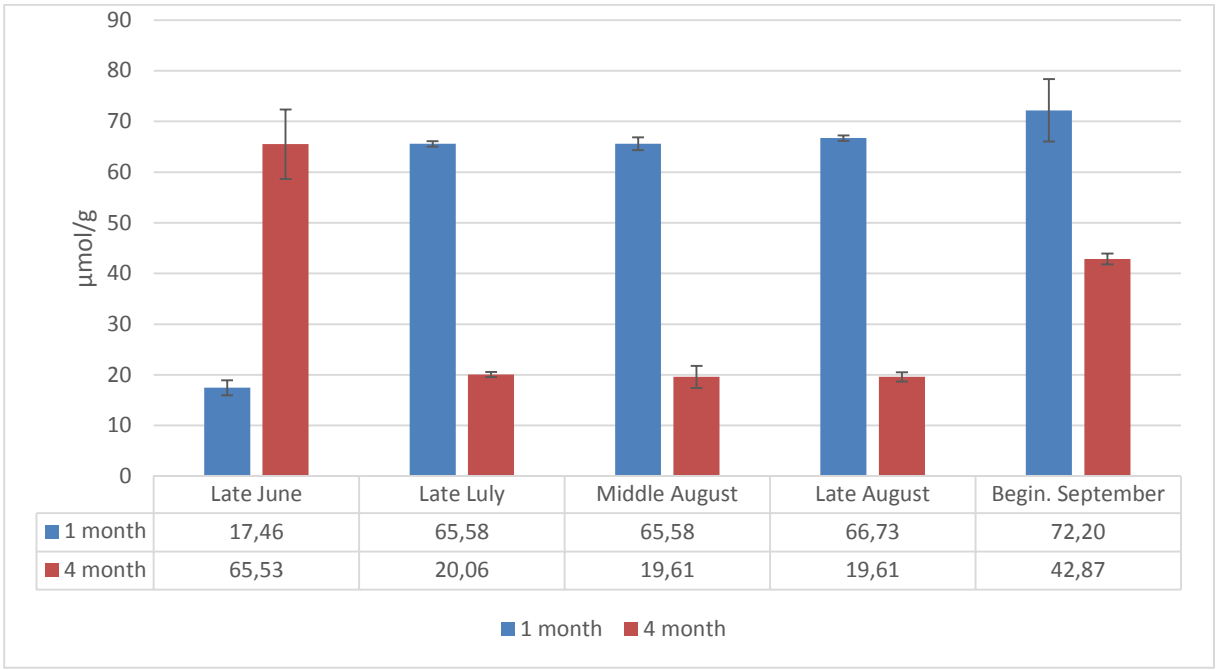
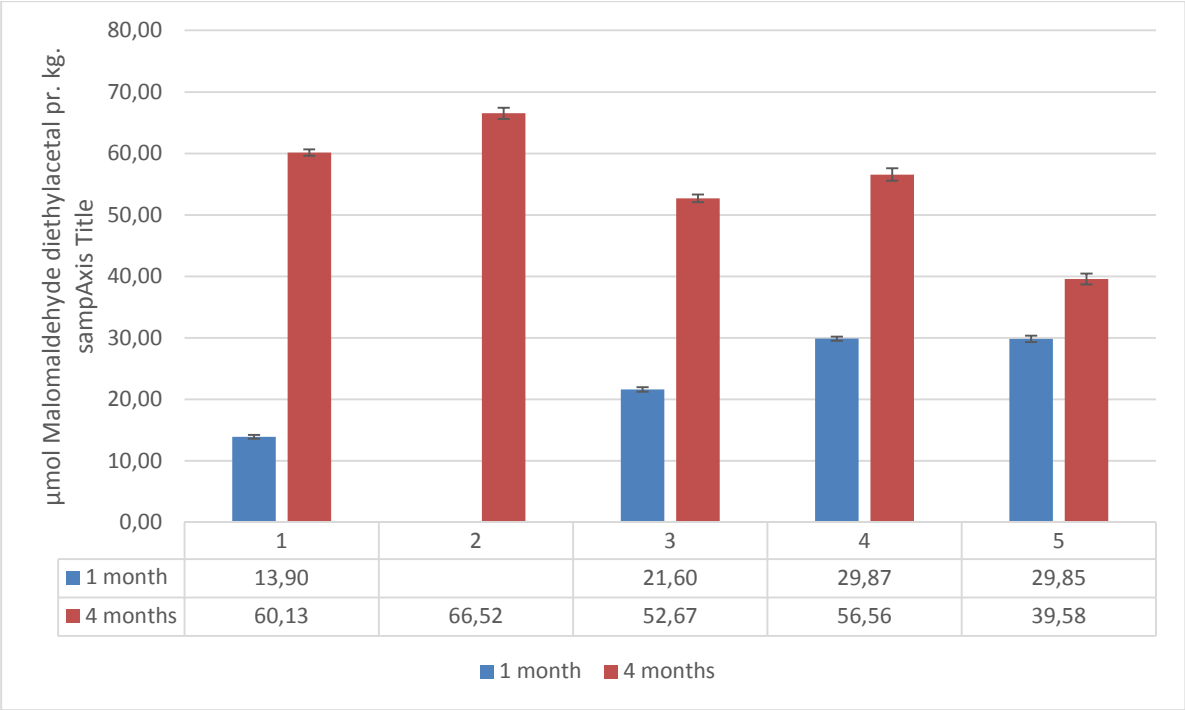


**Figure 4.2: Changes in peroxide values (µmol/g muscle) for all samples after 1 month and 4 months of storage at -27 °C**

For mackerel caught in late June, significant peroxide development was observed during the 4 months storage, from 17.46 µmol/g to 65.53 µmol/g. All the other samples showed different trend, significant decrease in the peroxide value during the storage time. Highest value after 1 month and 4 months storage were observed in beginning of September (72.2 µmol/g and 42.87 µmol/g).

### 4.2.2 TBARS

The TBARS values for the mackerel after 1 month and 4 months of frozen storage are shown in Figure 4.3. The same trend was observed for all samples. TBARS values increase from 1 month to 4 months of frozen storage at -27°C. Sample collected late July and stored for 1 month was lost; therefore, development of TBARS value for that period was not visible. Highest value of TBARS after 1 month of frozen storage were 29.87 µmol MDA/kg muscle in late August and lowest in late June 13.90 µmol MDA/kg muscle. After 4 months storage, the mackerel caught in late July had the highest TBARS value 66.5 µmol MDA/kg muscle and the lowest TBARS value after 4 months of storage was observed in mackerel caught in beginning of September 39.6 µmol MDA/kg muscle.




| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 month | 13,90 | | 21,60 | 29,87 | 29,85 |
| 4 months | 60,13 | 66,52 | 52,67 | 56,56 | 39,58 |

**Figure 4.3: TBARS values (µmol of MDA/ kg muscle) obtained from mackerel stored at -27 °C for 1 month and 4 months.**

### 4.2.3 FLUORENCE SHIFT RATIO

Increase in fluorescence shift ratio (δF) was observed for all samples during the storage as shown in Figure 4.4. Since samples stored for one month from late June are missing, it is difficult to evaluate if the same trend was for mackerel caught in late June. Lowest δF value after 1 month was 1,74 δF in late June and highest in late August 2.52 δF. Highest value after 4 months was 13.45 δF in late June and lowest 11.31 δF in beginning of September. No significant changes in δF values were measured after 4 months storage in all samples.

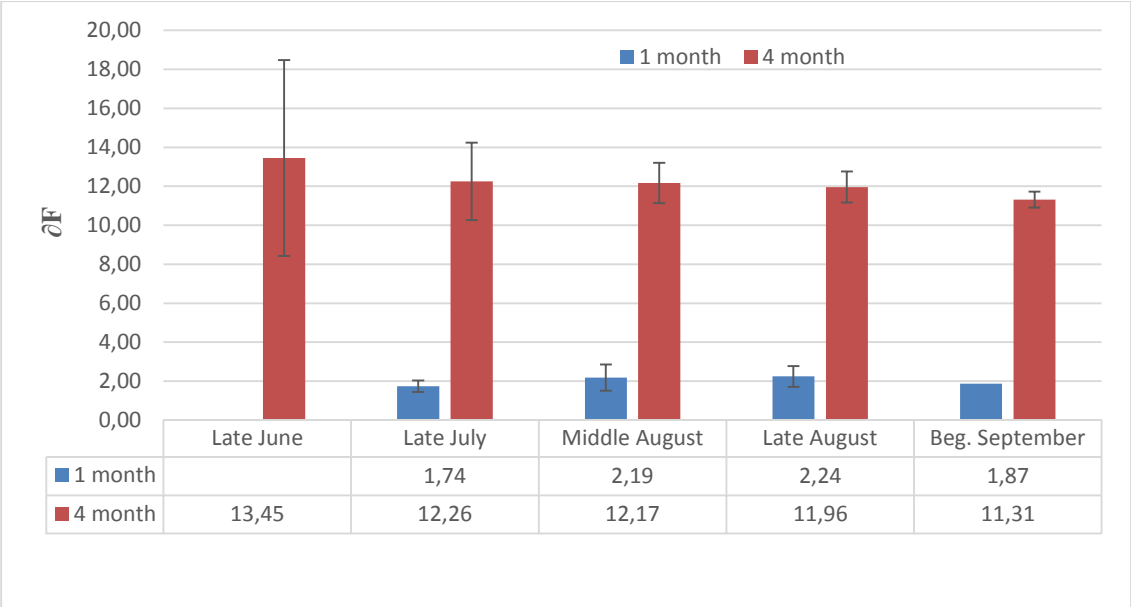



**Figure 4.4: Changes in fluorescence shift values (δF) for all samples after 1 month and 4 mounts of storage at -27 °C.**

## 4.3 LIPID HYDROLYSIS

Figure 4.5 shows results from free fatty acid (FFA) measurements after 1 month and 4 month of frozen storage. Results from late June samples after 1 month of storage are missing. Highest value after 1 month frozen storage were in late august 6.68 g FFA/100g lipids and lowest 4.37 g FFA/100g lipids. Highest value after 4 months frozen storage were 10.08 g FFA/100g lipids in late July and lowest 4.96 g FFA/100g lipids in beginning of September. Significant changes in FFA values from 1 to 4 months of frozen storage were measured in late July, middle of August and late August. Highest in late July 92% increase in g FFA/100g lipids value. In beginning of September, no significant changes from 1 month to 4 months storage were measured.

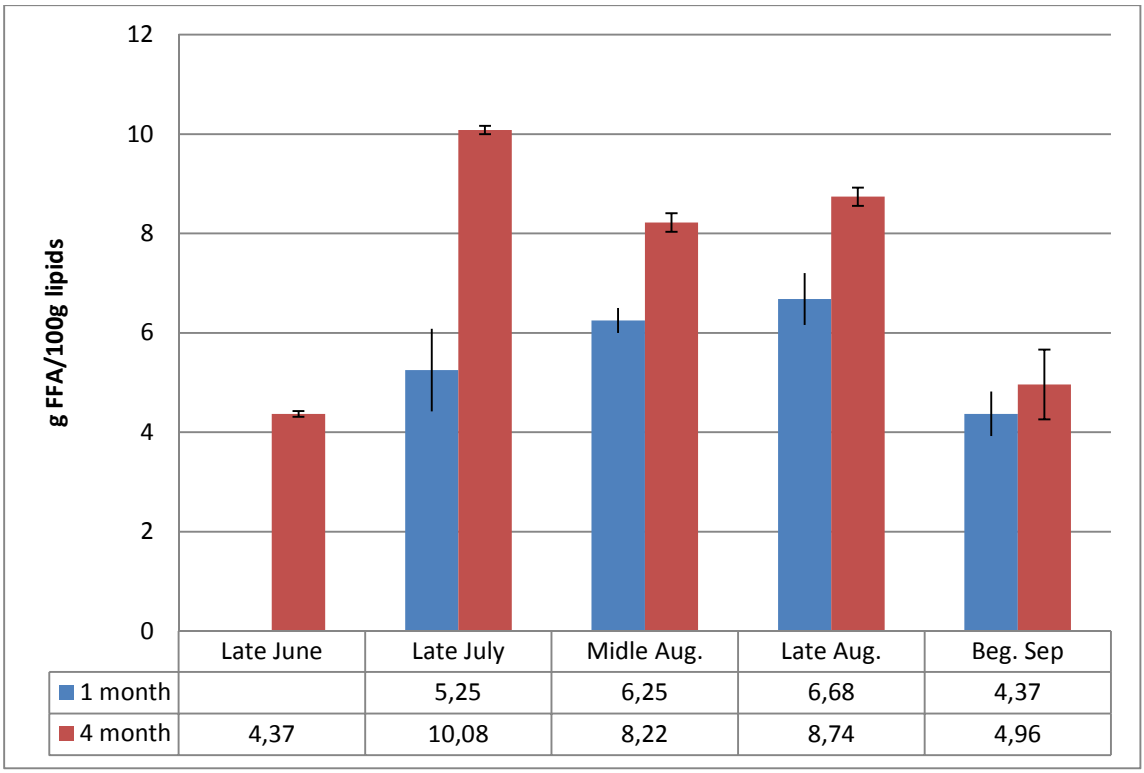



**Figure 4.5: Changes in free fatty acid values (g FFA/100 g lipids) for all samples 1 month and 4 months in storage at -27 °C.**

## 4.4 CORRELATIONS

In statistics, dependence is any statistical relationship between two random variables or two sets of data. Correlation refers to any of a broad class of statistical relationships involving dependence (Wikipedia 2015). In Table 4.5 correlations between oxidation products, hydrolysis, water and lipids are demonstrated. Those marked yellow are positive relations. These correlations were made between matching samples.

**Table 4.5: Correlation between oxidation products, hydrolysis, water and lipid.**

| | Water % | Lipid % | PV 1 month | PV 4 months | TBARS 1 month | TBARS 4 months | FFA 1 month | FFA 4 months | FL 1 month | FL 4 months |
|---|---|---|---|---|---|---|---|---|---|---|
| Water % | 1,000 | | | | | | | | | |
| Lipid % | -0,998 | 1,000 | | | | | | | | |
| *PV 1 month* | -0,884 | 0,866 | 1,000 | | | | | | | |
| *PV 4 months* | 0,926 | -0,943 | -0,814 | 1,000 | | | | | | |
| *TBARS 1 month* | -0,114 | 0,233 | -0,731 | -0,826 | 1,000 | | | | | |
| *TBARS 4 months* | -0,857 | 0,883 | 0,562 | -0,909 | 0,586 | 1,000 | | | | |
| *FFA 1 month* | -0,114 | 0,233 | -0,731 | -0,826 | 0,628 | 0,586 | 1,000 | | | |
| *FFA 4 months* | -0,857 | 0,883 | 0,562 | -0,909 | 0,586 | 1,000 | 0,586 | 1,000 | | |
| *FL 1 month* | 0,450 | -0,337 | -0,287 | -0,400 | 0,836 | 0,047 | 0,836 | 0,047 | 1,000 | |
| *FL 4 months* | 0,670 | -0,635 | -0,929 | 0,553 | 0,628 | -0,231 | 0,628 | -0,231 | 0,153 | 1,000 |

# 5 DISCUSSIONS

## 5.1 RAW MATERIAL AND FINAL PRODUCT

The biological stability was very poor in the beginning of the season when the mackerels were recovering from spawning and got better later in the season. This is as according to numerous studies (Huss 1995; Asthorsson *et. al* 2009; Sveinbjornsson *et. al.* 2008). The results from this study confirms that. The raw material from late June had significant feed damages and also skin damages.

The raw material used in this study came from the same fishing vessel. Fishing technique, handling on-board, landing, cooling practice etc. were therefore very similar for all the samples. One can assume that these factors are more or less constant and do not explain the differences in raw material properties, except when the cooling om board the vessel failed in the last sample. Biological factors are more likely to be responsible for observed variations in the quality of the raw material. It is important to notice that even when samples are taken from the same haul and tank the quality of the individual fishes can vary considerable. The first fishes entering the troll bag are in risk of getting crushed by fishes that enters later. That will result in quality reduction. Those fishes are also likely to die before being pumped on board and hence the spoilage rates are therefore already taken place. That depends on the hauling time and -speed for most parts. Haul size will also affect the quality. Unfortunately, that information was not available for this study. According to Isfelags requirements, limits are regarding haul sizes and time in order to avoid spoilage of fish in the troll bag. Therefor it can be assumed that to long hauling time and large hauls are not causing damages to the fish. But as observed the hauling time increased throughout the season parallel with better the mackerel getting stronger after being weak due to spawning. Something that is also according to Isfelag's requirements.

In late June the average size of the fish was much lower than later on in the season and the sea temperature high. According to Huss 1995, size and temperature are important factors affecting spoilage rate of mackerel. The average size of the mackerel was smallest for sample taken at the beginning of the season and the temperature of the sea was higher compared to later in the season. In general, smaller fish spoils faster than larger and spoiling rate increases with higher temperature.

As mentioned numerous times in this study temperature affects the spoiling rate significantly. The higher the seas temperature the longer the cooling down time. Temperature log showing how long time it took to cool down the fish to acceptable level was not available in this study so we cannot make any conclusions based on that.

## 5.2 FINAL PRODUCT (H/G MACKEREL)

For samples taken late July, middle of August and beginning of September, final product inspection showed results that reflected the raw material quality.
For late August final inspection showed lesser quality than indicated by the raw material inspection. That suggests that from the time of landing until it was processed spoilage took place in sample taken late August. One explanation for that is that on late August, the highest feed content was observed in the raw material something that affects the spoiling rate. In Isfelag all graded mackerel are put in tubs containing cooled brine, about -2 °C. The purpose of this brine is to keep the temperature of the fish as low as possible prior to processing. Eventually the temperature of the brine, and the fish, will increase, because the surrounding temperature is higher than -2°C. If the time from landing/grading and processing is to long spoilage will occur in the mackerel even though it is stored at low temperature. Temperature of the final product in the late August sample does not indicate that this happened so one must assume feed content is to blame.

During the process, removal of soft mackerel takes place to avoid soft mackerel from being packed and further exported. Low concentration of soft mackerel in late June final product compared to raw material inspection indicates that the removal process was successful. High concentration of soft mackerel in late August indicates that the removal process failed. However it is very difficult to remove all soft mackerel specially if it abundant in production.

The temperature of the fish caught in the beginning of September was much when landed than in any of the other samples. That indicates that something failed during the cooling on board. Even so that did not reflect on the final product. Suggesting that at the end of the season the mackerel is not as vulnerable as at the beginning.

## 5.3 CHEMICAL COMPOSITION AND TVB-N

Great variations in lipid and water content were observed as expected. Difference in protein content was a bit surprising. Reason for that were probably individual fish to fish variations. Seasonal variation in the lipid and water content was most likely due to changes associated with feeding and spawning of the mackerel. These results were however not entirely in agreement with previous studies (Huss 1995; Matis 2008) where the lipid content peaked in August. Different fishing grounds and individual variation in lipid content can explain this.

The freshness of raw material was measured by TVB-N. TVB-N is considered as useful parameter although it does not fully satisfy sanitarians. The samples taken were within the limit of what is acceptable (18-20 mg/100g) for human consumption of mackerel (Malle et al. 1983). The highest values were measured in the two samples from August, when the biological stability was higher than at the beginning of the season. That is probably because the fish in the August samples were 50-53 hours old when processed and analysed compared to 25-44 hours for samples taken earlier in the season. According to El Marrakcni *et. al*. 1990 TVN – B values in mackerel increases slightly during storage.

It is common practice to extent the mackerel fishing time at the end of the season when the fish is firmer and not as vulnerable. Keeping up the same fishing practice throughout the season would bring even better raw material quality later on in the season.

## 5.4 LIPID OXIDATION

**Peroxide value (PV) - Primary oxidation products**

Significant increase in PV was observed from 1 month to 4 months of frozen storage for samples collected in late June. That is a clear indication of that the primary oxidation had not reached its peak. However, considerable decrease occurred in all other samples. So it seems that sample from late June were not as oxidized as the others. One explanation for the increase in peroxide values can be related to the low lipid content in the late June sample compared to the other samples. It was positive correlation (0,866) between lipid content and PV oxidation after 1 month storage. No correlation was observed between lipid content and

PV after 4 months of storage. The lipid content was measured 15.1% in the beginning of the season (late June), resulting in the lowest content of all the samples. Aubourg *et. al.* (2005) observed that the lipid content of mackerel has an important effect on lipid oxidation development. Mackerel fillet (white muscle) from fish caught in November (lipid content 6.5-10%) showed significant higher changes in peroxide value after 4 moth of storage at -20 °C compared to mackerel fillets from fish caught in May (lipid content 1-3.5%).

There was a negative correlation between PV values measured after 1 month and 4 month of storage (-0,943).

Peroxide value measurements alone are not reliable in assessing the oxidation of highly unsaturated oils such as fish oils. This is because the peroxides initially formed are unstable and react quickly to form secondary oxidation products. For those reason the peroxide value should be used in conjunction with other methods (Saeed and Howell 2002).

**Thiobarbituric reactive substances (TBARS) – Secondary oxidation product**

Significant increase in TBARS during storage was observed for all sampling groups. For all samples, but late June, value of TBAR increased after 4 months storage when PV values decreased. Due to continuous lipid hydroperoxide breakdown resulting in production of secondary oxidation products. Highest values of TBAR after 4 months storage were observed in late July when lowest values of peroxide were measured, indicating that formation of secondary products were further in the process. There was a positive correlation between Lipid content and TBAR after 1 month (0,233) and after 4 months (0,883) of storage. In that correlation calculation, late June after 1 month is not present and therefore not included.

The results for TBARS for this study were similar compared to other studies, where significant increase was observed after 4 months of storage of Atlantic mackerel at -5°C, -10°C, -18°C, -20°C and -30°C (Saeed and Howell 2002; Aubourg et. al. 2005, Popelka et. al. 2012, Aubourg and Gallardo 2004).

Relatively high value of TBARS and PV after 4 month of storage in late June could indicate that despite production of secondary oxidation products amount of hydroperoxide still remained relatively high.

**Fluorescence shift ratio (δF) – Tertiary oxidation products**

Positive correlation between lipid content and fluorescence was observed after 1 and 4 moths of storage (0.45) and (0.67) respectively. Sample from storage after one months I late June are missing so it is not possible to compare low lipid content sample with those who have higher content after 1 month of frozen storage.

Result of comparable samples showed that storage time significantly affected the formation of tertiary oxidation products during four month storage. All the samples showed similar fluorescence values after 1 month and 4 months storage. According to previous studies (Aubourg et. al. (2005) fluorescence values for mackerel with high lipid values (caught in November) was low after one month of frozen storage and then increased during storage to the highest level after 4 months of storage. On other hand low lipid mackerel (caught in May) had higher fluorescence values after 1 month frozen storage and the increased slightly after 4 month storage. They argued that difference in nucleophilic compounds composition in the mackerel white muscle between May and November might be responsible for this.

## 5.5 LIPID HYDROLYSIS - FREE FATTY ACIDS (FFA)

Results for FFA showed that storage time led to an increase in FFA values. Most increase was measured in late July when lipid content was highest. There was a positive correlation between lipid content and FFA after 1 month storage (0,233) and after 4 months storage (0,883).

Results from Aubourg (2005) and Aubourg and Gallardo (2005), showed increase in FFA content for the first 4 months (13 g FFA/100g lipids) for mackerel caught in May and then slight decrease. For mackerel caught in November the FFA acid values changes very little. This mackerel was stored at -20°C and -18°C. According to Stodolnik *et. al*. (2005) mackerel caught in September had FFA content of below 1 gFFA/100g lipids for the first 4 months in storage at -20°C.

There was a positive correlation between FFA after 1 month of storage and 4 months of storage.

Compared with this study the difference can be because of higher temperature (-20°C) the FFA content increases sharper and there are higher values. Hwang and Regenstein (1988) found the temperature to be the only factor affecting hydrolytic rancidity in mackerel fillets.

According to studies made by Matis quality of mackerel diminish when FFA exceed 3-4%. All values in this study were over 4%, indicating that the overall quality of this mackerel was not good after 4 months of storage.

# 6 CONCLUSION

As expected, quality deterioration in form of lipid hydrolyses and oxidation took place in headed and gutted Atlantic mackerel during stored at about -27$^{o}$C for 4 months. The oxidation development varies, like the composition of the fish, throughout the season. Mackerel caught in July and August surpassed the phase of lipid oxidation faster, during frozen storage, compared to mackerel caught in June and September and had therefore higher oxidation rate. Higher lipid content in July and August mackerel was probably the main reason for that. Samples from the beginning of the season, when the mackerel had low lipid content and at the end of the season when the lipid content started to diminish after reaching its peak, had lowest oxidation rate and hence higher shelf life. According to this study it is reasonable to conclude that lipid content is the main contributor to quality declining in Atlantic mackerel during frozen storage other factors are less important.

Still lipid content is vital for allocation of the mackerel. Higher lipid content means access to markets who pay higher prices. Icelandic mackerel processors should therefore focus their fishing on August and September when the lipid content is optimal and they can maximise the value of the mackerel products. They should also consider lowering the frozen storage temperature in order to improve the shelf life and quality of the product.

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# APPENDIX A

This appendix describes were mackerel in the samples were caught (Figure A1).


**Figure A.1**: Fishing grounds were different samples were caught. Sample 1 (late June) and 2 (late July) south of Grindavik, sample 3 (middle August) and 4 (late August) east of Djupivogur and sample 5 (beginning September) east of Seydisfjordur.

# APPENDIX B

This appendix describes the quality inspection of raw material used in this study.

**Sampling** after landing and grading samples of whole mackerel, weighing 5-8 kg., containing 15-40 fishes, were evaluated according to the following descriptions.

1. Temperature taken by sticking a thermometer with needle into the thickest part of the muscle and recorded.

2. Each fish weighted, cut open and inspected for different parameters. Based on guidelines from (SINTEF 2012) and (BIM 2006)

Texture of whole fish, **soft** or **firm** muscle. Soft muscle indicates spoiling damages. Firm muscle indicates no visual- or minor damages.

**Table B.1:** Method of finding out if the mackerel is soft or firm.

| Parameter | Description | Grading |
|---|---|---|
| **Texture** | When pressing by the finger on the fish and it feels firm and the finger-mark is not visual | **Firm** |
| | When pressing with the finger the muscle feels soft and the finger-mark stays on. | **Soft** |

**Feed content** was evaluated visually in the mackerel intestine as described in Figure A4. For each sample, 10 fishes were cut open and the viscera gently pulled out. Then the stomach was taken and by visual inspection and by pushing the feed content gently to the end of the stomach with the fingers content of the stomach was evaluated.

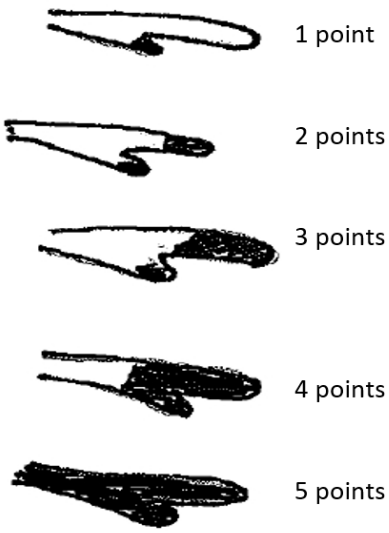


**Figure B.1:** Evaluation of the feed content in mackerel stomach.

**Calculation:** The total number of points for 10 fishes, divided with number of fishes.

**Skin damages** are all damages detected on the skin of the mackerel.

Example of skin damages are in Figure B.2.

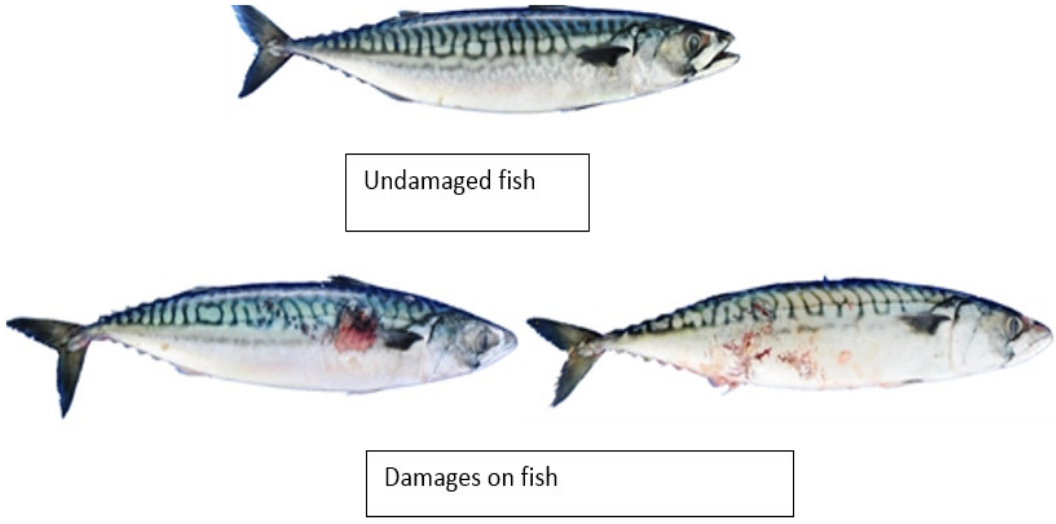


**Figure B.2**: Typical skin damages on mackerel, most often due to catching.

**Feed damages** are visual by cutting the fish open and inspect the muscle as shown in Figure B.3.


**Figure B.3:** Grading of peritoneum, 0 Peritoneum strong and firm, 1 peritoneum loose when touched, and 2 peritoneum is damages or none existing (SINTEF 2012).

Blood spots and damages spine are indication of rough handling during fishing.

# APPENDIX C

This appendix describes the quality inspection of fully processed H/G mackerel used in this study.

**Sampling:** Sample contains a whole block of mackerel (11 kg.). Samples were taken at the end of the production line (packing)

Temperature, average size, texture (soft and firm) were analysed using same method described in Appendix 2. Cutting defects, damaged and bad gut removal are all defects due to processing. Most occurring in the cutting machines.

Quality ratio was calculated by adding all the defects together, minus 100. For example quality ratio of 95% means that 95 % of the sample has no defects.

# APPENDIX D

This appendix describes the temperature fluctuations in storage for the complete period, Figure D.1.

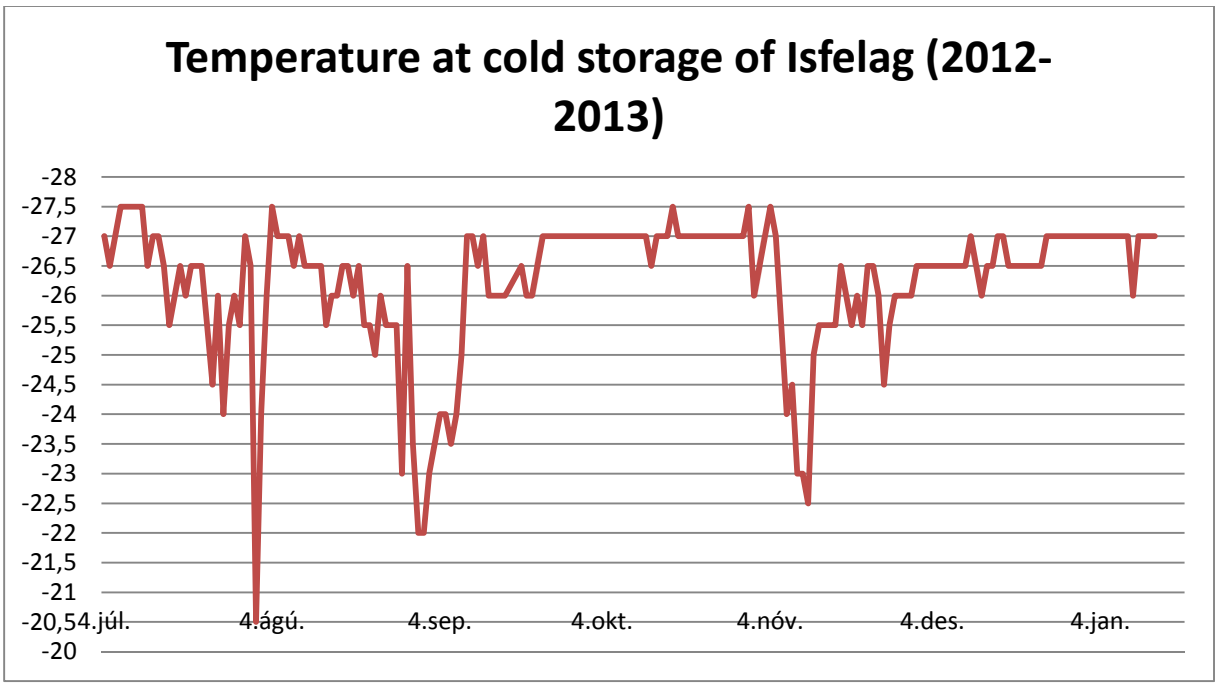


**Figure D.1:** Temperature in cold storage were all the samples used in this study where storage.