MUJEEB ADEWALE ADETOKUNBO

GRAVADING OF MACKEREL (*Scomber sp.*): PHYSICOCHEMICAL AND SENSORY CHANGES DURING REFRIGERATED STORAGE

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MUJEEB ADEWALE ADETOKUNBO

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Master's in Food Technology

Work carried out under the guidance of:

Prof. Eduardo Bruno Oliveira Esteves

Prof. Jaime Miguel Costa Aníbal

INSTITUTO SUPERIOR DE ENGENHARIA

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…….

MUJEEB ADEWALE ADETOKUNBO

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Abstract

The Atlantic Mackerel (*Scomber colias*) is a fast-swimming pelagic fish that can be found in the warm, temperate waters of the Atlantic Ocean, as well as on the shelf and upper slopes of the Mediterranean Sea. Mackerel is a great source of long-chain n-3 polyunsaturated fatty acids, compounds that have health benefits. Mackerel is also a good source of proteins, essential amino acids and other biologically active compounds. However, it is highly vulnerable to lipid oxidation due to the large amount of unsaturated fatty acids it contains.

Gravading is a fish processing technique popular in Scandinavian and Nordic countries, which involves applying a mixture of salt and sugar to the fish fillets and then letting it mature for 1-4 days at low temperature (in refrigeration) to produce gravads, a salt-sugar delicacy with a salt content of 3 –6% and a pH greater than 5. This process has been applied to fish such as salmon, whitefish, trout, or Greenland sole.

The main objective of this work was to study the effect of the gravading process on mackerel fillets using physicochemical, biochemical and microbiological parameters. Specifically, it was intended to study the effect on the quality and storage stability of mackerel that went through the gravading process; monitor some quality parameters, namely color (CIE Lab), texture (hardness), pH, water activity and moisture content, total volatile basic nitrogen content (TVB-N), or thiobarbituric acid reactive substances content (TBARS) and the presence/abundance of microorganisms evaluating total aerobic and psychotropic bacteria counts; and to study and understand how two different storage temperatures, 4 °C and 9 °C, can influence changes in these quality parameters.

Experimental and laboratory work took place from early April to mid-July 2021 at the Chemical and Food Processing Laboratories of the Department of Food Engineering of the Institute of Engineering (ISE) of the University of Algarve (UAlg), Campus da Penha, Faro, Portugal.

The fish, *S. colias*, were purchased from the Municipal Market of Faro, frozen on that date in an air-blast freezer under appropriate conditions (-25°C) and stored frozen (-18°C) until the beginning of the trial, in May 2021.

The quality parameters were evaluated for samples (n=3) of fresh fillets on the first day of sampling, before application of the application of the gravad mix, and after 48 h of gravading, the other fillets subjected to gravading were vacuum packed and divided into two groups, one set of packages was stored refrigerated at 4 °C and the other at 9 °C. The same parameters were evaluated in fillets (n=3) of each

storage condition, once a week until 4 weeks of storage, after which sampling was done once every two weeks until the 10th week of sampling, in July 2021.

The color parameters did not show a particular trend throughout the test, except in the case of L* luminosity, which decreased with time, probably due to the dehydration process. On the other hand, the water activity decreased to about 0.80, a level at which only yeasts and molds and halophyte bacteria can survive, contributing decisively to the stability of the product. The texture of mackerel underwent major changes due to gravading, as excess of sugar and salt increases the osmotic pressure and contributes to the removal of water from fish tissue. This caused the hardness to increase until the fourth week of storage, after which it starts to decrease. Generally speaking, quality parameters did not change with temperature, but all seem to deteriorate after four weeks. In fact, the TVB-N content gradually increased after the fourth week. TBARS values also increased during this period. The total aerobic and psychotropic bacteria counts were around 4 log(UFC/g) which is the common level for other fish species subject to gravading and well below 6 or 7 log(UFC/g), which is considered the critical level for microbiological contamination. This shows a high quality of raw material and product.

The fillets subjected to gravading, vacuum-packing and kept in refrigeration, regardless of storage temperature, appear to lose quality after four weeks, a shelf-life that can be partly credited to the vacuum packaging that inhibits the survival and growth of microorganisms.

Keywords: Mackerel, gravading, vacuum packaging, physicochemical parameters, microbiological contamination, spoilage.

Resumo

A cavala do Atlântico (*Scomber c*olias) é um peixe pelágico de natação rápida que pode ser encontrado nas águas quentes e temperadas do Oceano Atlântico, bem como na plataforma continental do Mar Mediterrâneo. A cavala é uma ótima fonte de ácidos gordos polinsaturados n-3 de cadeia longa, compostos que apresentam benefícios para a saúde. A cavala também é uma boa fonte de proteínas, aminoácidos essenciais e outros compostos biologicamente ativos. No entanto, é altamente vulnerável à oxidação lipídica devido à grande quantidade de ácidos gordos insaturados que contém.

Gravading é uma técnica de processamento de pescado popular nos países escandinavos e nórdicos, que envolve aplicar nos filetes de peixe uma mistura de sal e açúcar e, em seguida, deixar maturar por 1-4 dias a temperatura baixa (em refrigeração) para produzir gravads, uma iguaria com sal-açúcar com teor de sal de 3 –6% e um pH maior que 5. Esse processo foi aplicado a peixes como salmão, peixe branco, truta, ou linguado da Groenlândia.

O objetivo principal deste trabalho foi estudar o efeito do processo de gravading em filetes de cavala usando parâmetros físico-químicos, bioquímicos e microbiológicos. Especificamente, pretendeu-se:

Estudar o efeito sobre a qualidade e estabilidade de armazenamento da cavala que passou pelo processo de gravad;

Monitorizar alguns parâmetros de qualidade, designadamente a cor (CIE Lab), a textura (dureza), o pH, a atividade de água e o teor de umidade, o conteúdo em azoto (nitrogénio) básico volátil total (TVB-N), o teor em substâncias reativas ao ácido tiobarbitúrico (TBARS) e a presença/abundância de microrganismos, avaliando as contagens de bactérias aeróbicas e psicotrópicas totais; e

Estudar e entender como duas temperaturas de armazenamento diferentes, 4 °C e 9 °C, podem influenciar as alterações nesses parâmetros de qualidade.

O trabalho experimental e laboratorial decorreu de inícios de abril a meados de julho de 2021 nos Laboratórios de Química, Microbiologia e de Processamento Alimentar do Departamento de Engenharia Alimentar do Instituto de Engenharia (ISE) da Universidade do Algarve (UAlg),

Campus da Penha, Faro, Portugal.

Os peixes, *S. colias*, foram adquiridos no Mercado Municipal de Faro, congelados nessa data em congelador de ar forçado em condições adequadas (-25 °C) e armazenados congelados (-18 °C) até ao início do ensaio, em maio de 2021.

Os parâmetros de qualidade foram avaliados para amostras (n=3) de filetes frescos no primeiro dia de amostragem, antes da aplicação do gravad (gravading), e após 48 h de gravading. Os demais filetes sujeitos ao gravading foram embalados a vácuo e divididos em dois grupos, um conjunto de embalagens foi armazenado refrigerado a 4 °C e o outro a 9 °C. Os mesmos parâmetros foram avaliados em filetes (n=3) de cada condição de armazenamento, uma vez por semana até após 4 semanas de armazenamento, posto o que a amostragem foi feita uma vez a cada duas semanas até à 10ª semana de amostragem, em julho de 2021.

Os parâmetros de cor não apresentaram tendência particular ao longo do ensaio, exceto no caso da luminosidade L* que diminuiu com o tempo, provavelmente devido ao efeito de desidratação do processo. Por outro lado, a atividade da água diminuiu para cerca de 0,80, um nível em que apenas leveduras e bolores e algumas bactérias podem sobreviver, contribuindo decisivamente para a estabilidade do produto. A textura da cavala sofreu grandes alterações devido ao gravading, uma vez que o excesso de açúcar e sal aumenta a pressão osmótica e contribui para a remoção de água dos tecidos dos peixes. Isso fez com que a dureza aumentasse até a quarta semana de armazenamento, após a qual começa a diminuir. Genericamente, os parâmetros de qualidade não se alteraram com a temperatura, mas todos parecem deteriorar-se após quatro semanas. De facto, o teor em TVB-N aumentou paulatinamente após a quarta semana. Os valores do conteúdo em TBARS também aumentaram durante este período. As contagens de bactérias aeróbicas e psicrófilas totais, entretanto, ficaram em torno de 4 log(UFC/g) que é o nível comum para outras espécies peixe sujeita ao gravading e bem abaixo de 6 ou 7 log(UFC/g), que é considerado o nível crítico para contaminação microbiológica. Isso mostra uma alta qualidade da matéria-prima e do produto.

Os filetes sujeitos a gravading, embalados em vácuo e conservados em refrigeração, independentemente da temperatura de armazenamento, parecem perder qualidade após quatro semanas, um tempo de conservação útil que pode ser em parte creditado à embalagem a vácuo que inibe a sobrevivência e o crescimento de microrganismos.

Palavras-chave: Cavala, gravading, embalagem a vácuo, parâmetros físico-químicos, contaminação microbiológica, deterioração.

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1.0 INTRODUCTION

Fish. The name alone brings excitement. Fishes are healthy. This is mainly due to the presence of high-quality protein, iodine and a lot of vitamins and minerals. Fatty species, including Salmon, trout, sardines, tuna, and mackerel are considered the healthiest due to fat-based nutrients including Vitamin D, a nutrient lacking in multitudes. Omega-3 fatty acids which are essential for optimal brain function and stronger immune systems are present in huge amounts in fatty fishes. Vegans have also been known to take omega-3 supplements, made from microalgae, in lieu of eating fish (Budzko, 2018).

Fish and its derivative products are important for nutritional security because their consumption offers unique nutritional and health benefits. Aquatic foods have a unique function as a main source of the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Figure 1.1). DHA is a major component of the neural system, and as such, is a major player in the neurodevelopment in children and hugely important for full brain function. Omega-3 fatty acids can also be found in many vegetable oils, but they are mainly alpha-linolenic acid (ALA) which must be initially converted into DHA. Conversion of ALA into EPA and DHA in the body is usually inefficient, which is why reliance on vegetable oils is difficult especially during pregnancy and the first couple years of life. Pregnant mothers with fish in their diet therefore stand a better chance of delivering babies with optimal brain function (Budzko, 2018).

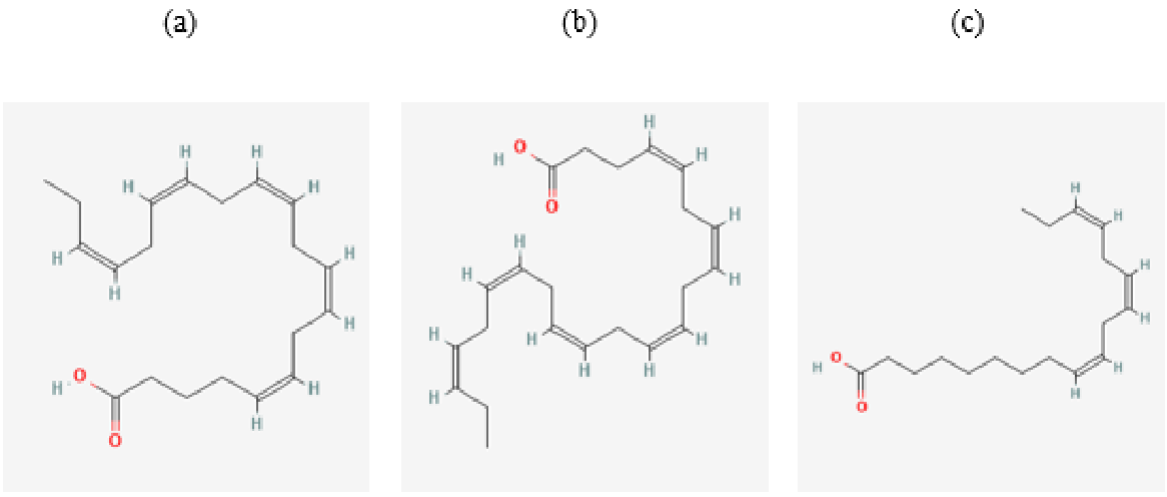

Figure 1.1 Illustrative, diagrammatic representation of (a) EPA, (b) DHA and (c) ALA (Pub Chem, n.d).

Fish and fishery products play an important role in food and nutritional security around the world. Consumption of fish offers unique nutritional and health benefits and is considered a key element in a healthy diet. Increased attention is given to fish as a source of essential nutrients in our diets, not only high value proteins, but more importantly also as a unique source of micronutrients and long chain omega-3 fatty acids (Budzko, 2018) (Table 1.1).

Table 1.1 Omega-3 fatty acids (mg) in 100g of fish (FAO 2018)

	ALA	EPA	DHA	Omega-3
Shrimp	6	68	70	138
Yellowfin Tuna	2	12	88	100
Tilapia	33	5	86	91
Salmon (farmed)	0	862	1104	1966
Catfish (farmed) cooked	0	20	69	89
Sea bass	0	161	434	595
Cod	0	64	120	184
Mussel	0	188	253	441
Clam	0	43	64	107
Eel	15	84	63	147
Herring	0	709	862	1571
Mackerel	0	898	1401	2299
Mullet	0	217	108	325
Nile perch	0	79	174	253

Fish and seafood products are often regarded as some of the healthiest foods available, as well as some of the least harmful to the environment. Fish waste consists of a wide range of materials, including damaged whole fish and non-edible parts such as viscera or skin, but these discarded materials have attracted attention in recent years, prompting researchers to develop methods to process them into useful products, taking advantage of their biochemical heterogeneity: lipids, proteins (or their hydrolysates), and polysaccharides are just a few of the molecules that can be obtained. Because of the high resale value of these byproducts, fish and seafood are a great tool for

protecting the environment while also benefiting the global economy. For these reasons, they are critical components of national, regional, and global food security and nutrition plans, and they play a critical role in reforming food systems and eradicating hunger and malnutrition.

Fish provided over 20% of the average per capita animal protein consumption for about 3.3 billion individuals. Fish accounted for over 17% of total animal protein and 7% of all proteins consumed Fish also deliver vitamins A, B, D, and many other minerals essential for various body tasks such as calcium, iodine, zinc, iron, and selenium, particularly when eaten whole. Increased fish consumption can help balance diets and fight obesity (FAO 2016).

The consumption of seafood and proteins differs by country, and it is predicted to rise in the future. Fisheries and aquaculture are important parts of the economy, providing jobs and income to millions of people. The seafood industry provides a steady flow of foreign currency to developing countries, as well as jobs, food, and nutrition. Fish exports brought in 80 billion USD for developing countries in 2014, more than all other industries combined (tobacco, rice, sugar). Global fish supply peaked in 2018 at around 20.5 kg per capita, whereas developed-country fish consumption peaked at 26.8 kg in 2013. Many countries' seafood trade is still expanding and developing countries account for more than half of all fish exports. Global fish production was estimated in 2018 to be 179 million tons, with about 156 million tons ending up on our plates (Figure 1.2). China, Peru, Indonesia, Russia, and the United States of America are the top manufacturers on the worldwide market, in that order (FAO 2018, FAO 2020).

The total amount of fish produced is predicted to increase from 179 million tons in 2018 to 204 million tons in 2030 (FAO 2020).

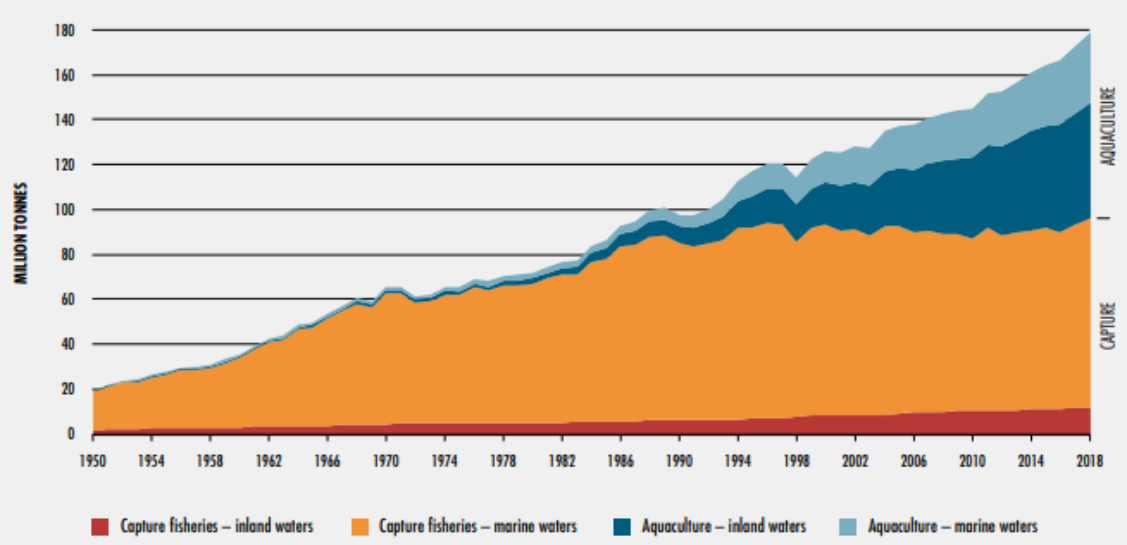

Figure 1.2 World fisheries capture and aquaculture production (FAO 2020).

In 2018, more than 156 million tons of fish were intended for direct human consumption, accounting for roughly 87 percent of world fish production (Figure 1.3). Fresh or chilled/frozen fish does not have to be consumed immediately. There are several processed items on the market, such as dried, smoked, cured, or salted (FAO 2020, Budzko, 2018). The remaining 23 million tons were used for non-food goods like fish oil, fishmeal, or as aquaculture feedstock (FAO 2018).

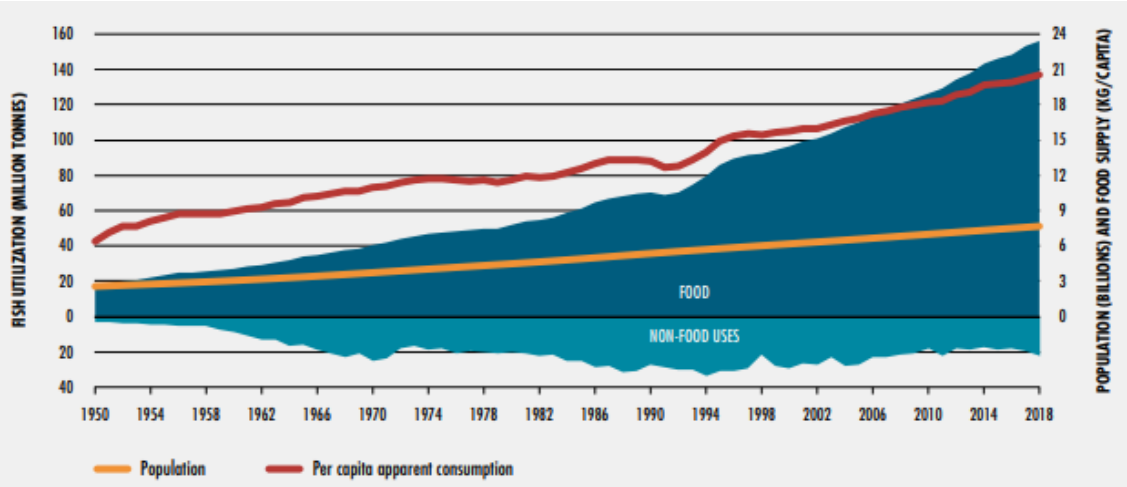

Figure 1.3 World's fish utilization and consumption (FAO 2020).

Of the 23 million tons for non-food goods, 80% (about 18 million tons) was mostly used as

decorative fish, culture (e.g., fry, fingerlings, or small adults for posterior growing), bait, pharmacological purposes, pet food, or as raw material for direct feeding in aquaculture and the cultivation of livestock and fur animals. From 67 percent in the 1960s, the proportion of fish consumed for direct human consumption has climbed dramatically. In 2018, live, fresh, or cold fish continued to account for most fish consumed directly by humans (44 percent), and was frequently the most popular and expensive type of fish. Frozen fish (35%) was followed by prepared and preserved fish (11%) and cured10% (10 percent). Freezing is the most common way of preserving fish for human consumption, accounting for 62% of all processed fish for human consumption (excluding live, fresh, or chilled fish) (FAO 2018).

Every year, more than 100 million tons of fish and shellfish are captured, and roughly 25% of this catch is wasted owing to microbial activity and chemical deterioration, accounting for 25% of overall agricultural and fishery product losses each year (Kaale et al., 2011). Fish and seafood are obviously healthful foods, but demand cannot be supplied by harvesting wild fish, mostly due to the world's finite fish populations. The amount of fish caught around the world increased from five million tons at the turn of the century to nearly one hundred million tons in the 1990s; furthermore, peak (steady-state) levels appear to have been reached around 1985, because the amount has remained constant since then despite constant improvements in fishing techniques (Cahu et al., 2004).

Aquaculture is the farming of aquatic animals such as finfish, crustaceans, and mollusks, as well as aquatic plants such as algae, in freshwater, seawater, brackish water, and inland saline water (FAO 2020). Aquaculture already accounts for about half of all fish consumed by humans, a figure that is expected to increase, making it the primary source of key nutrients provided by the fisheries sector. In 2018, aquaculture contributed 46.0 percent to worldwide fish production, up from 25.7 percent in 2000, and 29.7 percent in the rest of the world, excluding China, compared to 12.7 percent in 2000. Aquaculture accounted for 17.9% of total fish output in Africa, 17.0% in Europe, 15.7 percent in the Americas, and 12.7 percent in Oceania at the regional level. Aquaculture now accounts for 42.0 percent of Asian fish production (excluding China), up from 19.3 percent in 2000 (FAO 2020).

In 2018, world aquaculture production reached another all-time high of 114.5 million tons in live weight, with a total farmgate sale value of USD 263.6 billion (Figure 1.4) (The price of the product available at the farm, excluding any separately billed transport or delivery charge.) In 2030,

aquaculture production is expected to reach 109 million tons, up 32% (26 million tons) from 2018 (FAO 2020).

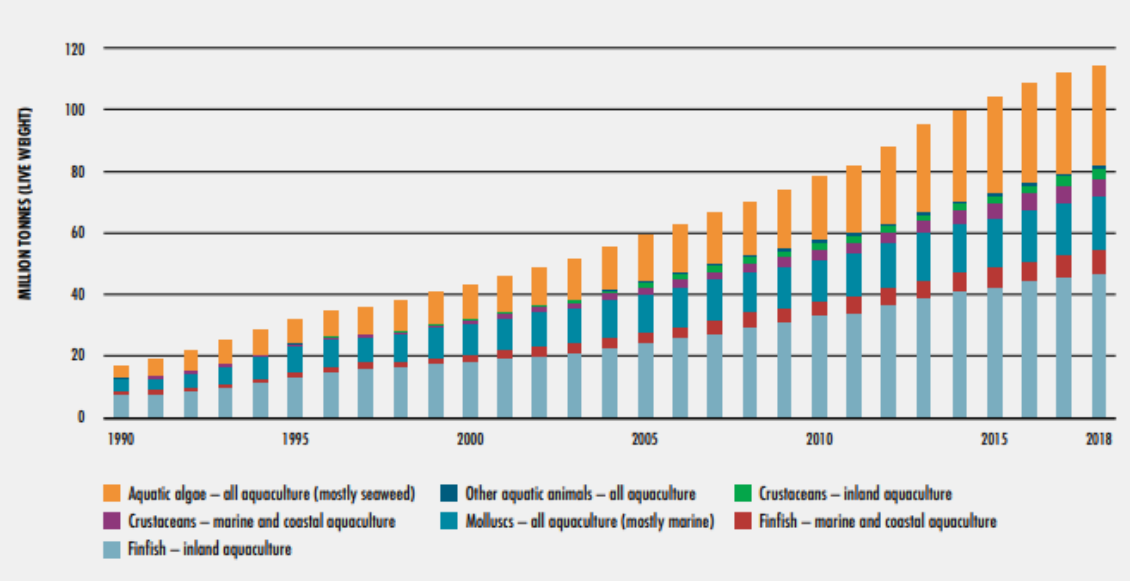

Figure 1.4 World's aquaculture production between 1990 and 2018 (FAO 2020).

The fisheries and aquaculture industries are a key source of employment all around the world. In 2018, the primary sector of fisheries and aquaculture employed an estimated 59.5 million people.

Aquaculture employed 20.5 million people, whereas fisheries employed 39.0 million, a modest rise from 2016 (FAO 2018).

Asia has the most fishermen and aquaculture employees, accounting for 85 percent of the global total. This implies that while fisheries and aquaculture is growing globally, the numbers are inflated by Asia (FAO 2018).

Even though the nutritional makeup of farmed and wild fish is nearly identical in most circumstances, there may be minor variances. The biggest nutritional difference between farmed and wild fish is related to the quality and quantity of fat. Farmed fish's nutritional composition is regularly compared to that of wild fish or other farmed fish. Farmed fish, on the other hand, should be compared to other farmed meats to demonstrate how aquaculture products have a distinct nutritional benefit by supplying high amounts of important elements, some of which are hard to come by in non-aquatic diets (FAO 2012).

Fish, both wild and farmed, are a healthier and more nutritious option to practically any other meat.

When opposed to their wild counterparts, whose environment, food, and access to food changes throughout the year, farmed fish have a more consistent nutrition composition. To ensure an optimal product, the environment of farmed fish can be monitored and maintained. Healthy fish and healthful fish products with the ideal nutritional composition can be obtained by adjusting the composition of aquaculture feeds and other inputs. Improved fishery resource utilization should also play a bigger role in satisfying the growing demand for valuable nutrients from the aquatic environment. Reducing post-harvest losses, which are estimated to be more than 10% in volume and up to 30% in value, might free up millions of tons of nutritious fish for human consumption (FAO 2011).

Despite the many advantages of marine products, there is one major disadvantage. Fresh fish, shellfish, and other aquatic goods must be consumed relatively quickly. They contain endogenous enzymes that encourage protein proteolysis in muscles and connective tissue. They can also encourage fat breakdown, which produces free fatty acids and aids lipid oxidation (Wu and Bechtel, 2008). Between 80-90 % of proteins in fish makes up the muscles, while the remaining are non-protein nitrogenous compounds such as volatile bases (ammonia, methylamine, dimethylamine, and trimethylamine), trimethylamine oxide (TMA-O), creatine, free amino acids (AA), nucleotides, purine bases, and urea, in cartilaginous fishes, all of which influence sensory properties and are involved in deterioration of fish and its by-products (Esteves et al., 2016).

Long-chain polyunsaturated fatty acids (PUFA) found in fat (or blue) fishes are nutritionally beneficial (e.g., eicosapentaenoic acid, EPA, 20:5n-3; docosapentaenoic acid, DPA, 22:5n-3; and docosahexaenoic acid, DHA, 22:6n-3) but very vulnerable to hydrolysis and oxidation (leading to rancidity) which leads to production of aldehyde and ketones which have particular smells. All these traits render fish and fisheries products particularly vulnerable to autolytic (A), microbiological (M), and chemical (Q) post-mortem deterioration. The myriad of symptoms includes the development of unpleasant tastes and smells (due to A, M, Q), the formation of mucus and the generation of gas (M), color changes/abnormal coloration (A, (M), Q), and texture changes (A, (M)) (Esteves et al., 2016).

A considerable amount of the global fisheries and aquaculture harvest is lost or wasted, accounting for 35 percent of the total harvest. Through suitable policies, regulatory frameworks, capacity building, services, and infrastructure, as well as physical access to markets, this must be decreased to increase the sector's efficiency and sustainability (FA0 2020).

The main objective of this work was to study the effect of the gravading process on mackerel fillets using physicochemical, biochemical, and microbiological parameters. Specifically:

- Study the effect on quality and storage stability of chub mackerel that has gone through the process of gravading
- Monitor quality parameters including color (CIE Lab), texture (hardness), pH, water activity and moisture content, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and the presence of microorganisms by evaluating the total aerobic mesophilic and psychotropic counts, and
- Study and understand how two different storage temperatures, 4° and 9°C, could influence the changes in those quality parameters.

2.0 LITERATURE REVIEW

2.1 ATLANTIC CHUB MACKEREL: BIOLOGY AND DISTRIBUTION

The Atlantic chub mackerel (*Scomber colias*) (Figure 2.1) is a fast-swimming pelagic fish that may be found in the Atlantic Ocean's warm and temperate waters as well as the Mediterranean Sea's shelf and upper slope. Chub mackerel can be found in the eastern Atlantic from the Bay of Biscay to South Africa, including the Canary, Madeira, Azores, and Saint Helena Islands, as well as a lot of seamounts (Castro-Hernández and Santana-Ortega, 2000). It is the southern congener of the Atlantic mackerel *Scombrus scombrus* (Villamor et al., 2004). In the Iberian Peninsula, the two species overlap, with Atlantic mackerel dominating to the north and chub mackerel dominating to the south of Lisbon (Martins et al., 2013).

Figure 2.1 Chub mackerel (Scomber colias) (World Register of Marine Species, 2019)

Based on mitochondrial and nuclear DNA analyses, *S. colias* is currently regarded a distinct species from the Indo-Pacific congener *S. japonicus* (Scoles et al.,1998, Collette, 1999, Infante et al., 2007). *S. colias* is referred to as chub mackerel, while *S. japonicus* is referred to as Pacific chub mackerel. Both species were formerly known as *S. japonicus*, and previous studies in the Atlantic and Pacific regions suggest they have a broadly similar life history and population dynamics. (Castro-Hernández and Santana-Ortega, 2000).

Chub mackerel is an early maturing species that can reach 50 cm in total length and 13 years of age. It grows up to 20 cm in the first year of life in Portuguese waters and matures at 1-2 years of age (Martins, 2007). Spawning occurs in the winter-spring, at temperatures ranging from 15°C to 20°C (Castro-Hernández and Santana-Ortega, 2000). Although the relative importance of larger species such as cephalopods, crabs, and small pelagic fish increases with the size of individuals (Castro and Hernandéz-Garca, 1995), both juveniles and adults feed mostly on zooplankton (Martins et al., 2013).

In several areas of the Pacific and Atlantic Oceans, migrations across latitude and between coastal and offshore habitats, linked to seasonal spawning and feeding cycles, have been described (Castro-Hernández and Santana-Ortega 2000). The locations of spawning sites and migration routes in European Atlantic waters remain unknown. The lack of genetic difference between the northeast Atlantic and the Mediterranean Sea refutes the presence of spawning grounds homing behavior (Zardoya et al. 2004).

Annual landings in the eastern Atlantic are estimated to be over 200000 tons, with 80 % taken off the coast of northwest Africa (FAO, 2010). Chub mackerel is mostly harvested in Portugal as part of the purse seine sardine fishery. Despite its poor economic worth, the chub mackerel is second only to the sardine in terms of total annual landings biomass and first-sale value (INE, 2011). Since 1986, chub mackerel landings in Portugal have ranged from 4000 to 23000 tons (compared to 727 tons and 40000 tons in previous years since 1958), fluctuating mainly inversely with sardine landings (Figure 2.2) (Martins et al., 2013). From the mid-1960s to the mid-1970s, landings were high, fluctuated at a low level (about 10000 tons per year) until the early 2000s, and then surged. Since then, chub mackerel landings have accounted for roughly 10% of all purse seine landings. Most of the landings are for human consumption in the form of fresh and tinned products (INE 2011). A minor proportion has been used for tuna feeding in recent years (Martins et al., 2013).

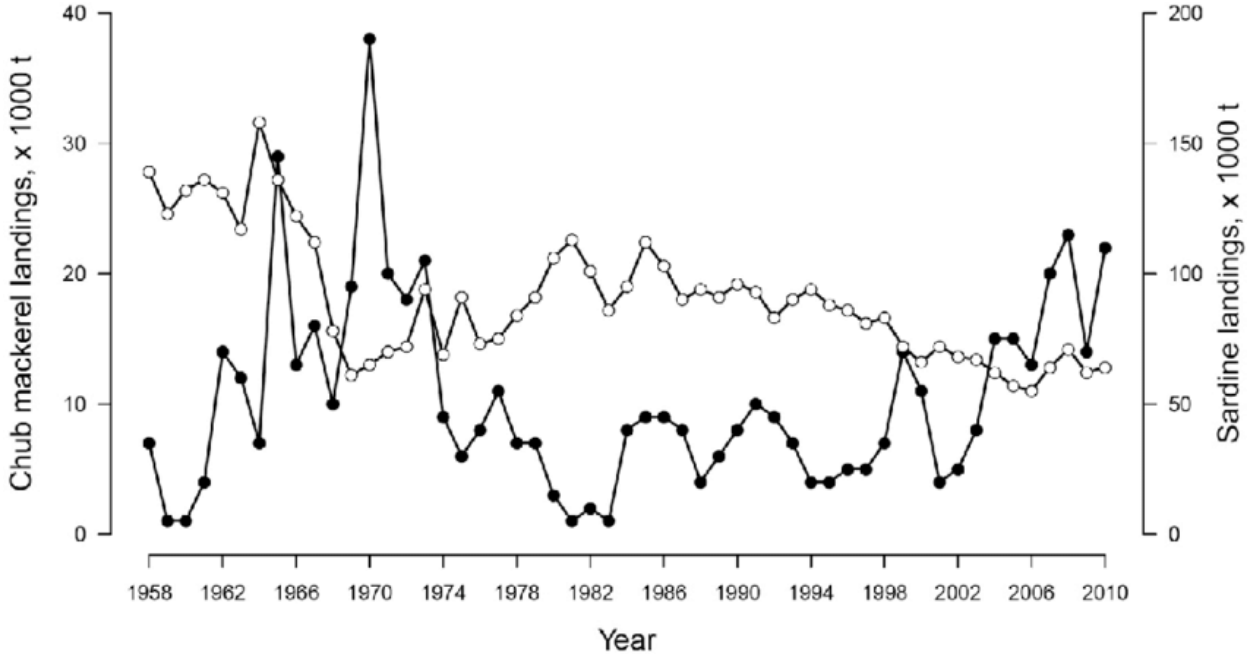

Figure 2.2 Landings of chub mackerel (filled circles) and sardine (open circles) in Portugal in years past (Martins et al., 2013).

Chub mackerel, like other small and medium-sized pelagic species, go through cyclical periods of abundance (Suda et al. 2008, Crone et al.2011). Temperature appears to play an important role in the recruitment success of chub mackerel. Higher temperatures, frequently associated with weak upwelling, are thought to be beneficial to chub mackerel spawning and productivity off South Africa (Crawford 1983), northwest Africa (Cury and Roy 1987), Japan (Yatsu et al. 2005), and the California Current System (Yatsu et al. 2005). At decadal timeframes in some locations, chub mackerel booms and bursts were part of worldwide changes generally related with environmental regime shifts affecting pelagic ecosystems (Castro-Hernández and Santana-Ortega 2000). In recent years, interest in chub mackerel has developed in Portugal, owing to fishermen's and scientists' perceptions of a simultaneous increase in its availability and decline in sardine abundance (Martins et al., 2013).

Mackerel contain high omega-3 polyunsaturated fatty acids (PUFA) content due to being fatty fishes but are also good sources of proteins, essential amino acids and other biologically active compounds (Budzko, 2018). Common nutrients in this species include 0.0046 mg/100 g fish of vitamin D, 0.020 mg/100 g fish of Iodine and 3550 mg/100 g of eicosapentaenoic acid (EPA) and 7-docosahexaenoic acid (DHA). Mackerels are considered safe to eat due to not containing large amounts of heavy metals or organic pollutants (POPs) (Budzko, 2018; Pazos et al., 2015).

Due to their high PUFA contents, mackerels are highly perishable and therefore needs efficient treatment to prevent and/or delay their deterioration. Storage shelf life depends on many factors, including temperature and compositional differences. Mackerels have been sold in different forms, the most common forms include frozen fillets, smoked mackerel, canned mackerel and those embedded in tomato sauce (Budzko, 2018; Arnasson, 2013).

2.2 MACKEREL FOR HUMAN CONSUMPTION

Mackerel is processed in several ways around the world, from smoked fillets to canned treats. Common mackerel products for human consumption include:

2.2.1 Smoked Mackerel

The process of smoking fish can be divided into two categories: cold smoking and hot smoking (Figure 2.3). The product is smoked at a temperature of up to 30 °C during the cold smoking process. In this method, significant thermal processing is avoided, and the structure of nutrients is conserved. As a result, cold smoking will not provide effective protection against dangerous germs, necessitating additional processing before consumption. The fish muscle is not cooked during the cold smoking process, and no protein coagulation occurs. Although the bulk of cold- smoked foods require additional heat processing before consumption, smoked salmon can be eaten right away (Bannerman 1980).

Cold smoking entails three steps: salting, drying, and smoking at a temperature of less than or equal to 30 °C (Montero et al., 2003). In Russia, cold smoked mackerel has a shelf life of 2-3 days. Cold smoked mackerel is usually canned before consumption (Thorleifsson, 2015).

The temperature of the product might reach up to 100 °C during hot smoking (Ünlüsayin et al., 2006). The fish is cooked because of the procedure, making it ready for immediate consumption. The flowchart in Figure 2.3 depicts a typical hot smoking technique for entire mackerel under mild conditions utilizing frozen raw material.

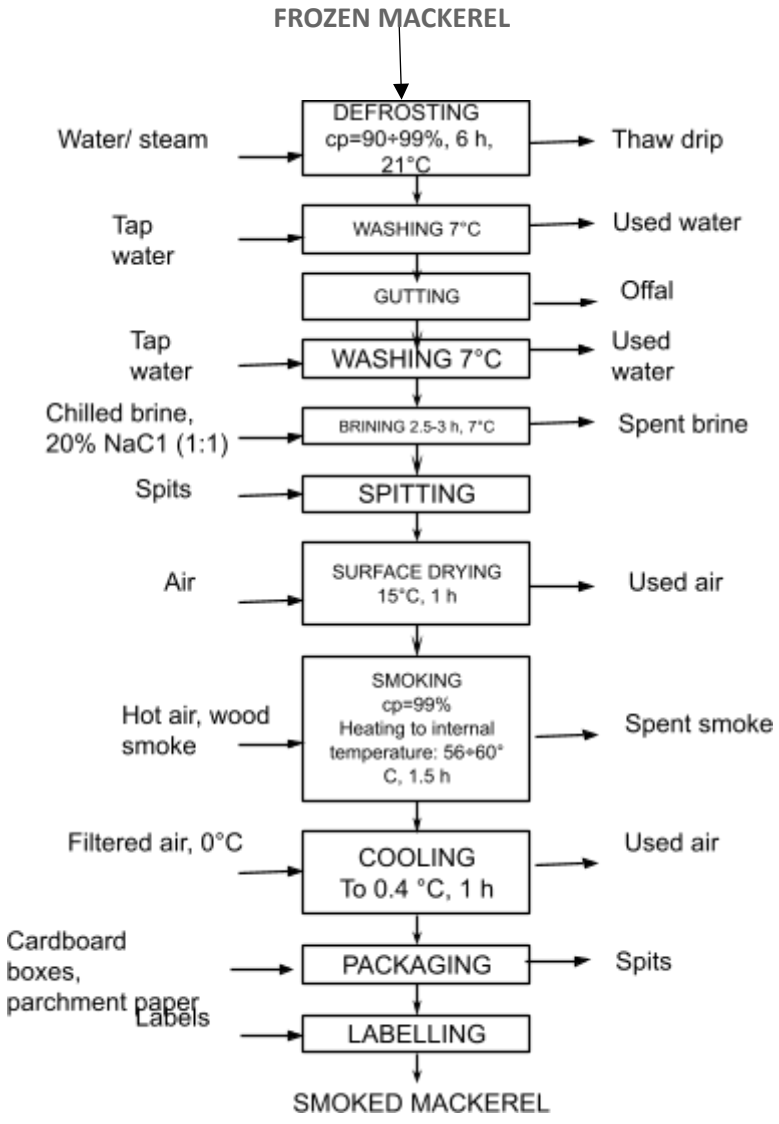

Figure 2.3 Chart of hot smoking of Atlantic mackerel (Stolyhwo et. al., 2004).

Smoking mackerel with a lipid content of at least 10% is preferred for an excellent eating quality product. Whole gutted fish, with or without the head on, or fillets can be smoked (Keay, 2001).

The entire smoking procedure should take about 3 h. The skin of the fish turns a dark golden-brown color and has a silky gloss. The meat should be totally cooked, with no jellylike flakes in the thickest region of the flesh at the shoulders of a whole fish (Keay et al., 2001). Products are exposed to a wide range of temperatures (40–100 °C) during the hot-smoking process, with the

temperature of the food's core point reaching up to 85 °C. Temperature, moisture, flow rate, and density of the smoke, as well as the water solubility and volatility of the specific compounds and the qualities of the fish surface, all influence the rate of deposition of distinct compounds (Ünlüsayin et al., 2006). In the case of temperature, an optimum value must always be determined to achieve the best deposition rates. Many smoke components have also been shown to interact with chemicals on the surface of smoked fish, boosting their deposition rates (Thorleifsson, 2015).

The success of the smoking approach can be influenced by several factors. The most important are raw material handling, process temperature, water activity, smoke qualities, and smoking duration (Goulas and Kontominas, 2005). The hot-smoking technique successfully combines thermal processing (heating and drying) with smoking, resulting in unfavorable conditions for the growth of dangerous bacteria and a reduced rate of oxidative reactions (Arvanitoyannis and Kotsanopoulos, 2011).

The antioxidant and antimicrobial characteristics of molecules ensure the food's preservation (Cornu et. al., 2006). For example, phenolic compounds produced by combustion, when paired with the temperature and smoking conditions, can inhibit microbial development and oxidation (Kjällstrand and Petersson, 2001). When woods are burned, substances like formaldehyde and phenols are released, giving smoking its preservation characteristics. During the smoking process, woods such as Ivory are frequently used to flavor the product and impart the characteristic organoleptic properties that this method bestow on smoked food. The temperature of the product must reach 62.8 °C, according to the US Food and Drug Administration (US FDA), and it must be maintained for at least for 30 minutes. Pasteurization of food is achieved in this manner, protecting it from hazardous germs (Huang et. al. 2002).

According to a study conducted by Jónsson and Sveinórsdóttir (2011), the yield from frozen raw material after brining and smoking was calculated to be 84 %, and the composition of hot smoked Atlantic mackerel was as follows: Water 53.4 %, Protein 19.9 %, Lipid 26.7 %, Ash 12.2 %, and Salt 0.18 %. The nutritional content of smoked food, on the other hand, is largely determined by the ingredients and state of the smoked fish (Huang et. al., 2002).

2.2.2 Canned Mackerel

One of the most important methods of preserving fish is canning. A chart of the general process of canning is shown in Figure 2.4. Many marine fish species generate excellent canned products, which play an essential role in human nutrition and have a recent yearly export value of over 2 million tons (FAO 2013). Because the meat of some marine species disintegrates under harsh thermal processing conditions, they are unable to adapt to canning. This is the case with some lean fish, which are made essentially unmarketable by traditional canning procedures due to their delicate flavor and structure (Aubourg, 2001). Mackerel steaks, fillets in a variety of sauces, and cold-smoked mackerel can all be canned (Thorleifsson, 2015)

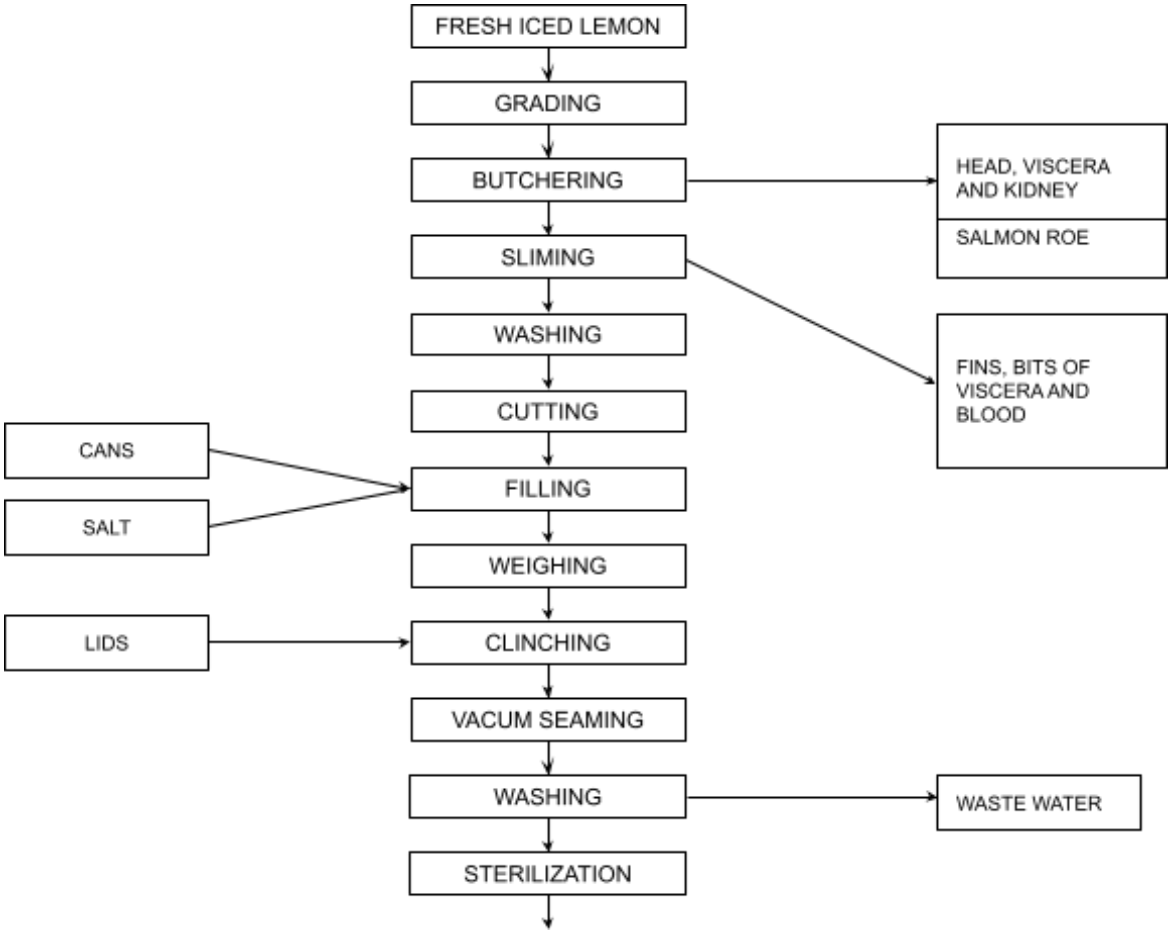

Figure 2.4 General process of fish canning (FA0 2010).

Due to water loss, there is a relative rise in lipid content after processing. The amount of moisture lost increases with the length of time the fish has been chilled, and fish with a medium fat content lose more weight and moisture than those with a greater fat level. 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 have all been observed during the cooking process prior to canning in terms of nutritional and sensory qualities, especially if over-processing is done (Thorleifsson, 2015).

2.2.3 Marinated Mackerel

Marinating is a food preservation method that entails treating muscles with solutions containing salt, spices, curds, lemon juice, and other ingredients, and it gives a wide range of muscle food products a high sensory acceptance (Yashoda et al. 2005). To increase the flavor and texture of the seafood, marinated fish is treated with acids (acetic or citric), salt, sugar, spices, and oil (Hwang and Tamplin, 2005). The marinating process reduces bacterial and enzyme activity, resulting in improved flavor, tenderness, textural, and structural changes as well as a longer shelf life (Sallam et al., 2007). This should be done at very low temperature as close to 0 °C as possible because marinating at room temperature can allow dangerous bacteria to grow and lead to foodborne illnesses (Szymczak et al., 2020).

In Japan, mackerel fillets are marinated in a variety of ways to produce a variety of goods. Shimesaba is one of these goods, with an annual production of 5000 to 6000 tons. Mackerel fillets are cured in 10% brine for 1 day and then immersed in marinated broth for 1 day to make this product. The broth is made up of varied ingredients that are unique to each producer, however it usually comprises 30 percent vinegar (Furutani et al., 2013).

2.2.4 Mackerel Patê

The flesh of a hot smoked mackerel can be used to make a superb patê. The flesh is thoroughly removed from the whole smoked fish, as are all bones. This can be done satisfactorily in a mechanical separator for commercial output. In a high-powered blender, combine the flesh with softened or melted butter, using seven parts by weight of fish to three parts butter. If needed, flavoring or spices are often added once the mixture has reached a smooth consistency; for example, lemon juice and pepper might be utilized. Manufacturers can easily come up with their own tweaks to the fundamental recipe. To compensate for fluctuations in the fat level of the mackerel, some white fish flesh is occasionally added (Keay, 2001).

2.3 FRESH MACKEREL SPOILAGE

Mackerel is rarely gutted at sea, so it spoils quickly unless it is chilled as soon as it is caught and kept refrigerated. Furthermore, because the muscle is soft and fatty, and the skin is not thick, fresh mackerel is more delicate to handle. Fresh cooled mackerel has a shelf life of 5-7 days (Xing et al., 1993), up to 9 days in ice, and 6–9 days for mackerel depending on the ice to fish ratio (Bennour et al., 1991). With undercooling (-2°C) and MAP, the shelf life can be extended up to 21 days (Thorleifsson, 2015)

The lipid content is also extremely important. Mackerel with a 10% lipid content start to give off odors after 1-2 days at 10°C, becomes soft and spoils quickly after 3 days, and becomes putrid after 5-6 days (Keay, 2001). Fish with a lot of lipids spoil even faster. The spoiling rate of fish taken during intense feeding and/or breeding seasons varies greatly. Fish that eat a lot of food are more prone to autolytic tissue degeneration than those who eat less. The type of feed/food that fish eat can also affect the rate of deterioration during storage.

Light feeding fishes have lower quantities of germs in their stomachs than heavily feeding fishes, according to Huss (1995). Previous study has revealed a considerable endogenous pro-oxidant activity (Saeed and Howell, 2001) as well as quality loss during frozen storage (Jia et al. 1996) and further processing (Huss, 1995; Saeed and Howell, 2001). The quality and shelf life of fish are also affected by fishing technique, fishing area, handling procedures, preservation, storage period, temperature, and the qualitative and quantitative composition of fish microflora (Thorleifsson, 2015).

2.3.1 Autolytic Enzymatic Spoilage

The digestive enzymes in mackerel are quite active, and they attack the walls of the digestive canal almost immediately after capture. Rough handling renders the tissues considerably more vulnerable to rupture (Huss, 1995). The effects and prevention of enzymes in the digestive tract of fish are summarized in Table 2.1. Autolysis is caused by stomach enzymes. It causes the meat to soften, the abdominal wall to tear, and the blood water, which includes both protein and oil, to drain (FAO 2005).

Much of the influence is on textural quality, as well as hypoxanthine and formaldehyde production. Autolytic enzymes, according to Hansen et al. (1996), impair textural quality at early stages of

deterioration but do not cause the typical spoilage off-odors and off-flavors. This suggests that even with moderate quantities of spoilage microbes, autolytic degradation can limit shelf life and product quality. When a mackerel's belly is full of food, the belly is particularly vulnerable to tissue degeneration, which can result in a disease known as "belly burst," in which the fish's neck develops a dark discoloration (Thorleifsson, 2015).

Table 2.1 Summary of changes in chilled or frozen fish (FAO 2005 adapted from Thorleifsson, 2015).

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 ^{o}C, physical abuse, freeze/thawing

2.3.2 Oxidative Spoilage

For pelagic fish species with high lipid content, lipid oxidation is a key cause of deterioration and spoiling (Fraser and Sumar, 1998). Lipid oxidation in foods is a complicated chain of processes that starts with primary products (peroxides) and then progresses to secondary oxidation products, which include aldehydes, ketones, epoxides, hydroxyl compounds, oligomers, and polymers when exposed to protracted oxidation conditions. Most secondary oxidation products have unpleasant sensory and biological effects (Kanner, 2007). As a result, maintaining control over it is critical.

Initiation, propagation, and termination of free radicals are the three stages involved in lipid oxidation. Initiation is the generation of lipid free radicals by catalysts such as heat, metal ions, and irradiation. Peroxyl radicals are formed when free radicals combine with oxygen. Peroxyl

radicals combine with other lipid molecules to create hydroperoxides and a new free radical during Propagation (Fraser and Sumar, 1998). When a buildup of these free radicals interacts to generate non-radical products, Termination happens. The reaction of oxygen with the double bonds of fatty acids is known as oxidation. As a result, polyunsaturated fatty acids in fish lipids are especially vulnerable to oxidation. To allow oxidation to take place, molecular oxygen must be activated. Transition metals are the principal molecular oxygen activators (Hultin, 1994).

Peroxides, particularly hydroperoxides, are the first chemicals generated during the oxidation process, and are thus referred to as primary oxidation products. Hydroperoxides are frequently subjected to further oxidation, resulting in secondary oxidation products. Aldehydes, ketones, epoxides, hydroxy compounds, oligomers, and polymers are among the many secondary oxidation products. There are both volatile and non-volatile molecules among them, with hexanal and malondialdehyde (MDA) serving as main representatives respectively (Thorleifsson, 2015**;** Ikape, 2017).

Shahidi and Spurvey (1996) explains that the value of thiobarbituric acid reactive compounds (TBARS) in fresh mackerel increased over time. This is because lipid hydroperoxides continue to break down and form secondary oxidation products during storage. According to studies, storage luminous compounds are produced during food processing as a result of the reaction of primary and secondary oxidation products with biological amino compounds (Thorleifsson, 2015).

2.3.3 Lipid Hydrolysis

Free fatty acids (FFA) are produced when lipid hydrolysis occurs. FFAs are either relevant in terms of oxidation products or have been shown to have a direct sensory influence. Lipid hydrolysis is depicted in Figure 2.5 where FFA are produced. The enzyme (triglyceride lipase) from the digestive tract cleaves triglycerides in the depot fat (Huss, 1995).

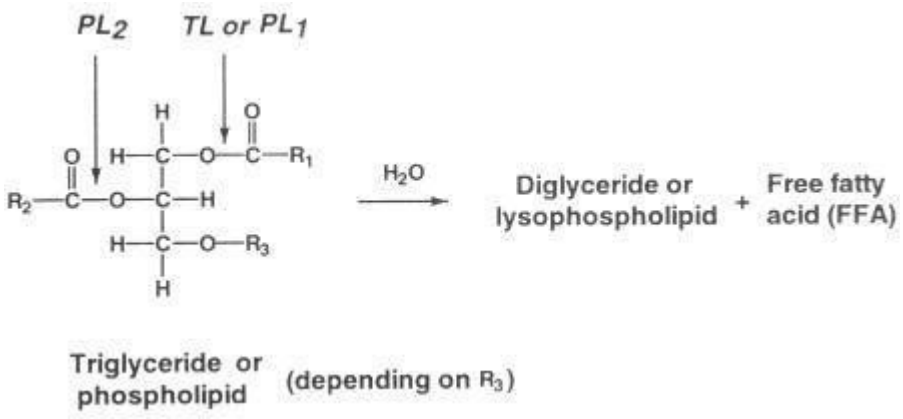

Figure 2.5 Triglycerides and phospholipids primary hydrolysis (Huss, 1995).

In fish and fish products, lipid hydrolysis is a prevalent post-mortem characteristic. Endogenous and/or bacterial lipolytic enzymes are involved, with the main products being free fatty acids (FFA) and glycerol. Free fatty acids are almost nonexistent in the fat of living animal tissue, but they can occur after the animal has been killed. Phospholipids, triacylglycerols, cholesterol esters, and wax esters are the most easily hydrolyzed (Vladimiros, 2005). Lipid hydrolysis is negligible in chill-stored eviscerated fish; nevertheless, significant FFA concentrations can develop during storage of whole, round fish, especially at high temperatures. Triacylglycerols in the depot fat of fattier species are cleaved by triglyceride lipase from the digestive tract or expelled by specific microbes. Due to a larger diffusion of digestive enzymes from the viscera of rotting fish, as well as the intervention of bacterial lipases, lipolysis can be more severe during the later phases of storage (Vladimiros, 2005).

Because polyunsaturated fatty acids (PUFAs) linked to phospholipids at glycerol-carbon atom 2 are more vulnerable to oxidation than fatty acids esterified to glycerol, hydrolysis may result in enhanced oxidation. However, the link between lipolytic activity and oxidation remains a mystery. Some researchers claim that lipid oxidation happens more quickly in FFA-rich tissue, while others claim that FFA derived from phospholipids inhibits lipid oxidation in muscle tissue (Thorleifsson, 2015; Vladimiros, 2005). The impact of lipolysis on the acceptability of fish and fish products is still unknown. The fatty acids themselves may have a “soapy” taste to them. Even though the terms rancid and soapy are frequently used as descriptors in tasting panel score sheets of chilled and frozen storage fish, no link between the development of rancidity or soap-like flavors and fatty acid production appears to have been established. Fatty acids and/or soaps formed by lipid

hydrolysis may interact with proteins, influencing the fish's textural properties (Vladimiros, 2005).

2.4 BRIEF OVERVIEW OF METHODS OF ANALYSES

2.4.1 Color Parameters

In the food and bioprocessing industries, color is an important quality factor that determines consumer choice and preferences. The chemical, biochemical, microbiological, and physical changes that occur during development, maturation, postharvest handling, and processing determine food color. Because it is simpler, faster, and correlates well with other physicochemical qualities, color measurement of food products has been employed as an indirect indicator of other quality attributes such as flavor and pigment content (Pathare et al., 2012).

The CIELAB L*, a*, and b*, which was created in 1948 for photoelectric measurement, and the CIE L*a*b* color space, which was established in 1976, provide consistent color differences regarding human perception. The presence of color necessitates the presence of an object, a light source or illuminant, and an observer. A light source can be used to view an object and can be turned on and off. An illuminant, on the other hand, is a mathematical description of a light source.

The L*, a*, and b* CIELAB coordinates were read directly. It was referred to as the CIELAB uniform space, and it was used to measure two color coordinates, a* and b*, as well as a psychometric index of lightness, L*. The parameter a* is positive for reddish colors and negative for greenish colors, whereas the parameter b* is positive for yellowish colors and negative for bluish colors. L* is a rough measure of luminosity, which is the quality that allows each color to be considered comparable to a member of the greyscale, which ranges from black to white (Granato and Masson, 2010).

The quantitative feature of colorfulness, chroma (C*), is used to assess the degree of difference between a hue and a grey color of the same luminance. The color intensity of samples perceived by humans is proportional to their chroma values (Pathare et al., 2012). The following equation was used to calculate chroma:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \qquad 2.1$$

Hue angle (h*), a qualitative characteristic of color, is the attribute by which colors are historically classified as reddish, greenish, and so on, and it is used to define the difference between a given

color and a grey color of the same luminance. This characteristic has to do with the absorbance differences at different wavelengths. In assays, a higher hue angle indicates a less yellow character (Pathare et al., 2012):

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad 2.2$$

A red hue is represented by an angle of 0° or 360°, whereas yellow, green, and blue hues are represented by angles of 90°, 180°, and 270°, respectively. It has been widely used to assess color factors in green vegetables, fruits, fishes, and meats (Barreiro et al. 1997; Lopez et al. 1997).

The total color difference (ΔE) between the stored and control samples indicates the magnitude of the color difference (Patras et al. 2011):

$$\Delta E = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}} \qquad 2.3$$

2.4.2 Texture

A compression test can be used to estimate the maximum force required to deform the sample's surface (Esteves et al., 2021). It is the simplest and most widely used method of determining texture instrumentally. A sample is put on a flat base/surface and a flat probe/platen is pushed onto the sample to a given force or distance in the simplest approach (or the sample is compressed to a percentage of its original height). The sample is deformed, and the extent of the deformation and/or the sample's resistance are measured. Uniaxial compression refers to simple compression tests in which the sample is compressed in one direction but unconstrained in the other two. A sample can be compressed to a low or high degree, but a high degree of compression will usually cause it to rupture, spread, fracture, or split into pieces (Stable Micro Systems, n.d).

Compression testing is used in a variety of sectors to ensure the strength, quality, and performance of components, as well as the compressive qualities of materials and completed products. Compression tests give objective quantitative data on the integrity and safety of materials, components, and manufactured goods, allowing you to determine whether an item is suitable for its intended purpose and will not fail during storage, handling, transportation, or usage (Stable Micro Systems, n.d).

Because most foods are viscoelastic rather than elastic and are frequently subjected to high compressions during testing, the formal definition of Young's modulus rarely applies to food

materials, thus the term "modulus of deformability" may be more suitable. However, under somewhat light compressions and in the area of the force-compression curve that is reasonably linear, the concept of Young's modulus of elasticity is usually utilized to express the food's stress-strain ratio (Stable Micro Systems, n.d).

2.4.3 pH

In the canning and seafood processing sectors, measuring the pH of seafood is critical since it corresponds with its freshness and can be used to determine its shelf life. Fresh seafood has an average pH of 6.2, though this varies depending on the type of fish. Scientific studies have discovered a direct link between the pH of seafood and its freshness.

It is well recognized that seafood is a perishable raw material, and that enzymatic and microbial changes affect its shelf life and integrity during storage (cooling or freezing) and transportation (under the same conditions). As a result, adequate procedures for preserving its quality and freshness are required. The rate at which each of these changes occurs is determined by how basic conservation principles, hygiene, cold chain maintenance, as well as the species taken and the means of capture, were used (Goncalves, 2017).

The hydrogen ionic potential, i.e., pH, has the function of indicating the acidity or alkalinity or neutrality of seafood muscle in an aqueous medium. The determination of pH represents an important factor in the quality evaluation of various foods, such as seafood, which is considered a food of low acidity (pH higher than 4.5). The concentration of the hydrogen ions is almost always changed when the hydrolytic, oxidative, or fermentative decomposition of muscle is processed the higher pH and bacterial activity. However, it is not conclusive as the only parameter to evaluate the degree of freshness of the seafood, and chemical, microbiological, microscopic and/ or sensorial analyses should be performed to have greater reliability in the results (Goncalves, 2017).

2.4.4 Water Activity and Moisture

Food science specialists employ water activity in foods for product development, quality control, and food safety. It also became a key criterion for assessing and monitoring food safety and quality. When it became clear that water content could not fully account for microbial growth restrictions, the use of water activity as a tool in quality and safety measurements was first recommended in

the 1950s. As a panel of experts on water activity decided in 2000, dividing water into categories defined as free bound or available water is incorrect since water activity describes the continuum of energy levels of the water in the system. A moisture sorption isotherm curve is a non-linear connection between water activity and moisture content (Figure 2.6) (Abbas et al., 2009).

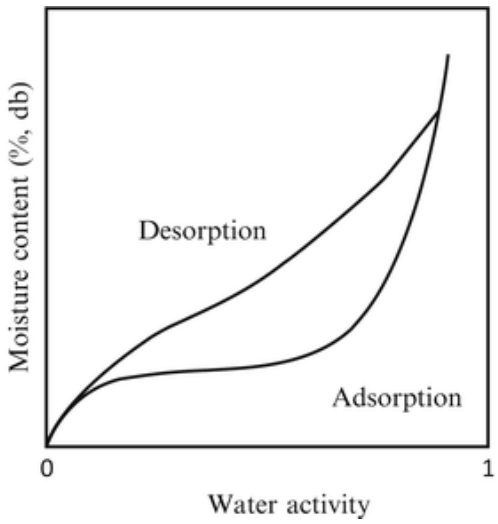

Figure 2.6 The relationship between water activity and moisture content (Caballero-Ceron et al., 2015).

These isotherms are particular to the substance and temperature. Isotherms can be used to forecast product stability over time in a variety of storage environments. Every microorganism has a minimum, optimum, and maximum water activity for growth, much like pH does. Yeasts and molds can thrive in low-water environments (Figure 2.7). However, for pathogen growth, 0.85 is regarded as a safe cutoff level. Most fresh fish, fruits, and vegetables are examples of moist foods (those with water activities above 0.85) since they have a relatively high water activity. Only because of the numerous barriers of pH, water activity, and mold growth, which is preferred over pathogen growth, is it safe (Abbas et al., 2009).

The microbiological and chemical stability of fish and fish products is determined by the product's water activity (a_w). Most bacteria can't survive in media below 0.9 water activity, but Staphylococcus aureus can survive down to 0.85 water activity. The rate of microbial growth, as well as the range of microorganisms that can grow, is higher in freshly caught fish, with water activity exceeding 0.95. This can be reduced by drying and salting operations, which reduce the rate of microbial growth as well as the range of microorganisms that can grow. The water activity content of fish products has a big impact on their microbiological and chemical stability throughout processing and storage. Microbiologically stable fish and fish products have a water activity of

less than 0.6 (Abbas et al., 2009).

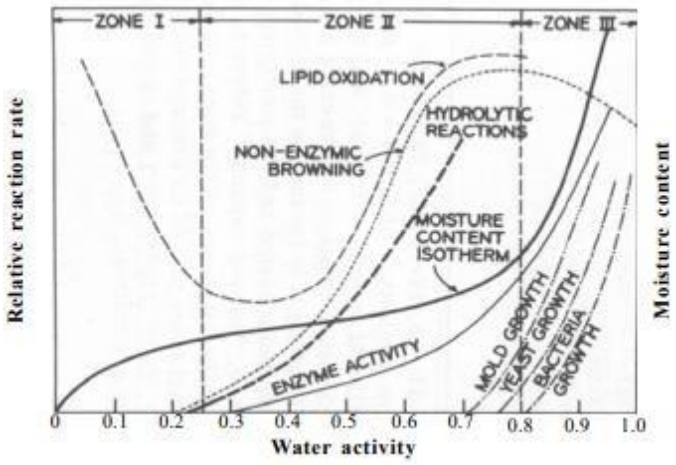

Figure 2.7 The influence of a product's water activity on different types of reactions (Abbas et al., 2009).

2.4.5 Thiobarbituric acid reacting substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) are generated as a by-product of lipid oxidative damage (i.e., as fat degradation products) and can be detected using the TBARS assay (which uses thiobarbituric acid (TBA) as a reagent). TBA interacts with malondialdehyde (MDA), a low-molecular-weight end-product generated by the decomposition of several primary and secondary lipid peroxidation products. TBA data may be misleading since not all peroxidation reactions produce MDA. MDA isn't the only by-product of fatty peroxide production and decomposition, nor is it a chemical produced solely by lipid peroxidation (Dorsey and Jones, 2017).

Previously, the thiobarbituric acid (TBA) test was assumed to be primarily a measure of malondialdehyde (MDA); however, it is now known that TBA reacts with a variety of chemicals, referred to as thiobarbituric acid reactive substances (TBARS). Compounds such as 2-alkenals and 2,4-alkadienals, as well as MDA, can create the 532 nm red pigment produced by interaction with 2-thiobarbituric acid. Measurement of a yellow pigment at 450 nm (TBARS 450), has been used in studies such as in the analysis of freeze-dried beef (Irwin and Hedges, 2004; Havens et al., 1996).

TBARS content is a secondary lipid oxidation indicator, used typically in meat and fish products. Direct extraction or distillation are the most common methods for performing the procedure. In the work of Irwin and Hedges (2004), maceration of the sample in a 7.5 % solution of

trichloroacetic acid (TCA) is used in the direct extraction method, which is applicable to raw meat and fish. An aliquot of the extract was reacted with TBA in a boiling water bath for 35 minutes after it has been filtered. After cooling, the solutions were compared to standards made from 1,1,3,3 tetraethoxypropane and read against the reagent blank. TBARS was calculated by plotting malondialdehyde versus absorbance and expressed as mg MDA/kg. A modified version of this procedure is used for our experiments on TBARS in this thesis and will be described in the methodology part. The distillation process can be used to analyze cooked meats or samples with a high fat content where turbidity is a concern. An aliquot is distilled at a regulated rate until a predetermined volume is collected after extraction in TCA and centrifugation. After that, a portion of the distillate is taken for TBA reaction. The recovery of MDA using this method will be incomplete, hence it's critical to distill the standards as well. The distillation method can also be utilized on lipid extracts from meat or fish samples, as well as oil and fats (Irwin and Hedges, 2004).

TBARS, especially in meat and fish products, can be a strong indicator of rancidity. Other compounds found in foods, such as sugars, acids, esters, amino acids, and oxidized proteins, may also react with TBA. Because the fatty acid content of a dietary sample will influence the actual level of TBARS that may occur, comparing data from different sample types should be done with caution.

2.4.6 Total Volatile Base Nitrogen (TVB-N)

Volatile amines are the molecules responsible for the fishy odor and flavor in fish several days after they've been caught, and they're frequently employed as a criterion for judging fish quality. Freshly caught fish muscle contains an average of 10 mg/100 g wet weight of ammonia, which

increases during refrigerated storage due to endogenous and bacterial enzyme deamination activities. Ammonia, according to some researchers, is a poor indicator of seafood freshness and should not be used as a reliable indicator of fish rotting (Paarup et al., 2002; Altissimi et al., 2018).

Enzymatic and bacteriologic activity can rapidly reduce the protein content and quality of stale seafood; ammonia, trimethylamine, dimethylamine, and other volatile basic nitrogenous compounds, collectively known as TVB-N, are formed (Fallah et al., 2015; Wu and Bechtel, 2008). Total volatile basic nitrogen (TVB-N) content is a key criterion for evaluating the quality of

seafood items and is one of the most common chemical indicators of marine fish deterioration (Amegovu et al., 2012; Wu and Bechtel, 2008; Altissimi et al., 2018).

Various amounts of biogenic amines are produced during the decomposition of seafood, depending on the fish species, and have been proposed as a freshness indicator of fish products, including cuttlefish and crustaceans (Lehane and Olley 2000; Prester et al., 2011). Histamine, tyramine, putrescine, and cadaverine are the most prevalent biogenic amines linked to seafood deterioration (Lehane and Olley, 2000). Bacteria naturally found in decomposing fish decarboxylate the equivalent free amino acids to produce them (Onal, 2007; Altissimi et al., 2018).

2.4.7 Total Aerobic and Psychrophilic Bacterial Counts

The total aerobic and psychrophilic aerobic bacterial counts were carried out according to the NMKL standard no. 184 (NMKL, 2006) by surface spreading.

This method is a routine technique to monitor aerobic and specified spoilage organisms in fish and fish products. For the determination of aerobic plate counts of fresh and lightly preserved seafood, Long & Hammer agar is used. The Long and Hammer (LH) culture medium contains peptones, which are amino acids and polypeptides necessary for the development of microorganisms, gelatin, dipotassium phosphate, sodium chloride, agar with a gelling function, and distilled water (NMKL, 2006).

Spread plating on Long & Hammer Agar allows detection of psychrotolerant and thermally labile micro-organisms. These microorganisms include the CO_2-resistant *Photobacterium phosphoreum*, which often dominates the spoilage of fresh marine fish, especially in vacuum and modified atmospheric packaging. In fresh minced fish and mildly preserved fish, psychrotolerant and thermally labile microorganisms can also dominate microflora (NMKL, 2006).

Long & Hammer agar (spreading plating) aerobic count at 15°C covers every colony which grows aerobically on the medium's surface when testing is carried out in the way described in this procedure (NMKL, 2006).

Luminous colonies can be detected on Long & Hammer agar, but the medium is not discerning for these bacteria. *P. phosphoreum* colonies may be bioluminescent, but frequently, only a small fraction of *P. phosphoreum* from fresh modified atmosphere packaged fish is luminous. In fresh

fish, *P. phosphoreum* can be precisely detected by using a conductance-based detection technique (Dalgaard et al. 1996). *P. phosphoreum* is thermally labile and will die in 45 °C hot agar, which is why it cannot be detected by Iron agar pour plating (NMKL, 2006).

Quantitative evaluation of the aerobic viable count at 15 °C is executed on the surface of Long & Hammer agar, incubated aerobically at 15.0 ± 1.0 °C for 5-7 days. Luminous colonies can be counted after 4 days in a dark room (Dalgaard et al., 1997).

2.5 GRAVADING

Universally, there is an increasing interest for minimally processed fish with increased shelf life, like food varieties of marine origin, which are alluring for diet variety. Their worldwide appeal is constantly expanding. Increasing customer request driven by changes in dietary patterns in recent years has roused the fish business to build up an assortment of convenience foods including ready to eat or prepare fish items. It is in this way fundamental that these products should be taken care of accurately. The issue associated with such food, however, is its perishability. To improve the shelf life of minimally processed foods, the use of natural food additives is especially proper (Pankyamma et al., 2020; Rzepka et al., 2013).

Minimally processed products of the pickled, salted, or cold-smoked type make up most fishery products. A less known ready to eat (RTE) food product is gravads, a salt-sugar delicacy hugely associated to Scandinavian and Nordic countries. Eaten without further heating, they belong to the ready-to-eat products and are characterized by a salt content of 3–6% and a pH greater than 5. In Finland, several fish species are used as raw material including salmon (*Salmo salar*), whitefish (*Coregonus lavaretus)*. and rainbow trout (*Oncorhyncus mykiss*) (Lyhs et al., 2001). Traditional Scandinavian gravaded products are obtained primarily from salmon, trout and Greenland halibut but also from mackerel and herring sometimes (Michalczyk and Surowka, 2007).

Gravading technology involves rubbing fish fillets with a salt-sugar mixture, and then maturing for 1–4 days at base temperature. Gravads can be consumed without further thermal processing since they are ready to eat (RTE) products. As such, gravading is an essential preservative processing technology because it reduces water activity and inhibits spoilage microorganisms. It also encourages membranous surfaces which prevent subsequent growth of microorganisms (Durmuş et al., 2017; Michalczyk et al., 2009).

Gravads may be famous in Nordic nations as RTE delicacies but may not be seen as such in Southeast Asian and African nations. It therefore can be marketed as ready to cook product instead. In the process of gravading, determining the optimum fish to gravad mixture (sugar-salt mixture) for the aspired quality is very important. Salt level, past the required amount might lead to muscle shrinkage and protein loss. Expansion of sugar improves the surface, grant great taste, and extend the shelf life but a high level of sugar, past the optimum level, is also not desirable as it increases the sweetness of the final product (Pankyamma et al., 2020).

Fat content and fillet structure are important factors for gravading (Lyhs et al., 2001), which is why mackerel, being a fatty fish is a potentially good raw material for gravading.

2.6 PACKAGING OF FISHERY PRODUCTS

2.6.1 Vacuum Packaging

Vacuum packaging (VP) is considered one of the most popular pre-storage packaging for fish products. In vacuum packaging, the product is enclosed in a package constructed of a low-oxygen-permeability material that is sealed airtight after the air has been vacated, using a vacuum sealer (Figure 2.8a). Food vacuum packages are simple to handle. The growth of aerobic spoilage organisms is inhibited by vacuum packaging. Due to the oxygen barrier features of the packing material, removing oxygen from the packets slows down oxidative reactions in the food during storage. Vacuum packages are clear, oil resistant, chemical resistant, and operate as an oxygen and vapor barrier. In addition to ensuring product safety, vacuum packaging lowers the economic loss of stored fish and fisheries products (Budzko, 2018; M. Michalczyk et al., 2008).

With vacuum packaging, the shelf life of vacuum-packed products can be extended for periods ranging from six days upwards. When compared to air-packed samples, vacuum packaging was much more successful at preventing microbial deterioration. Even though the product may not develop rancid flavors over time, it may develop unpleasant scents and flavors as a result of bacterial activity. Vacuum packaging with preservatives and storage at a chilled temperature could be utilized to extend the shelf life of vacuum-packed products significantly (Lyhs et al., 2001; Patil et al., 2020). The many processes involved in gravading technology puts the products at increasing risk of microbial contamination which makes it high risk since the products are usually eaten without cooking. This, combined with the shortfall of vacuum packaging mentioned earlier highlights the need for microbiological and spoilage research of vacuum-packaged gravad products, something there is not a lot of literature about.

VP is a type of modified atmosphere packaging (MAP) (see next) in which the maximum quantity of air from a packed seafood product is evacuated. By restricting the availability of O_2, which is required for the growth of aerobic bacteria, VP paired with cold storage enhances the shelf life of marine products. Furthermore, VP has an acceptable moisture and gas permeability, allowing for assembly and protection against contamination from the outside environment (Esteves et al., 2021).

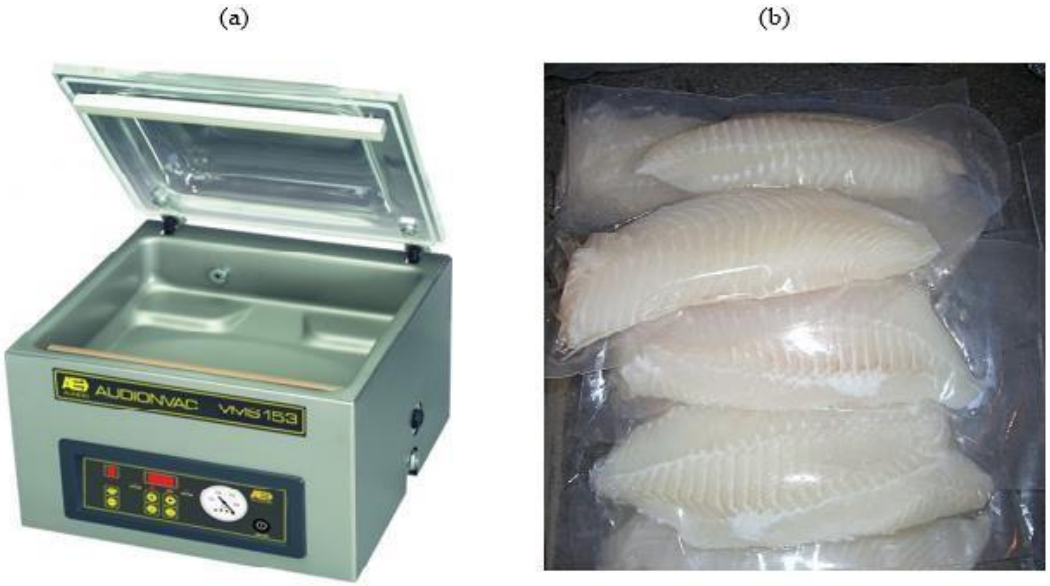

Figure 2.8 (a) A typical vacuum sealer for food packaging (Pac Machinery, n.d). (b) Vacuum packaged fish fillets (Food Safety Magazine, 2014).

2.6.2 Modified Atmosphere Packaging (MAP)

Another packaging method in fish processing is modified atmosphere packaging (MAP). As a supplement to ice or mechanical refrigeration, MAP has been developed as a scientifically viable approach to reduce losses and increase the storage lifetime of fish and shellfish products. The popularity of MAP packaged foods has grown in response to consumer desire for fresh, refrigerated goods with a long shelf life. It's a technique for extending the shelf life of perishable items such as meat, fish, fruits, and vegetables by slowing the natural decay of the food with the aid of machine designed for the purpose (Figure 2.9a).

Figure 2.9 (a) A simple MAP machine (alibaba, n.d) (b) A typical modified atmosphere packaged seafood product (Worldfishing, 2015)

The air surrounding the food within the package is replaced with different gas mixes in modified atmosphere packaging preservation techniques to regulate microbial activity and/or postpone discoloration of the contents (M. Michalczyk et al., 2008; Swain & Mohanty, 2020). The gas blends mostly used include nitrogen, carbondioxide and oxygen, with the sole purpose of extending the product's shelf life beyond that of ordinary chilled storage. During the logarithmic phase, CO_2 inhibits microorganism development by increasing the lag phase and slowing the rate of development. When food is stored at chilled temperatures, carbon dioxide inhibitors are more effective. MAP is used with a variety of products, and the gas mixture inside the package is determined by the product type, packaging materials used, and storage temperature. Non-respiring

items such as meat, fish, and cheese require very low gas permeability and high barrier films, but fruits and vegetables are respiring items that require the packaging material to interact with the food product. By raising CO_2 and decreasing O_2, the rate of respiration and ethylene generation is slowed, spoilage is delayed, and different ripening compositional changes are delayed. The main principle behind MAP is to replace the air in the package with a different preset gas mixture (Swain & Mohanty, 2020).

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

The experimental, laboratorial work took place from the beginning of April to the middle of July 2021 in the Food Processing and Chemistry Laboratories of the Food Engineering Department of the Institute of Engineering (ISE), University of the Algarve (UAlg), Penha Campus, Faro, Portugal.

The fishes, *Scomber colias* were procured from the Faro Municipal Market on the 7th of April 2021, frozen on that date in blast-air freezer under suitable conditions (-25 °C and 8 m/s airflow) and stored frozen (-18 °C) until the beginning of trial on the 4th of May 2021.

Quality parameters including color, texture, pH, water activity, contents in total volatile base nitrogen (TVB-N) and thiobarbituric acid reactive substances (TBARS) were carried out on fresh, gravaded and then stored gravaded mackerel fillets, first to determine the effect of the process of gravading on mackerel fillets and then the effect of different storage temperature and time on gravaded fillets. Microbiological parameters of total aerobic and psychrophilic bacteria were also evaluated. All the parameters were evaluated for fresh fillet samples on the first day of sampling before the remaining fillets were gravaded. Three of the ripened gravaded sample fillets were also evaluated for the same parameters after 48 h. The remaining gravaded fillets were vacuum packed in fours and divided into two groups, one set of packages was stored refrigerated at 4 °C and the other set at 9 °C. The same parameters were also evaluated for two packs of fillets, one from each storage condition, once every week until after 4 weeks of storage in which sampling was done once every other week until sampling concluded on the 15th of July 2021. Microbiological parameters were not evaluated for the last week of sampling. Figure 3.1 describes the experimental design of the study.

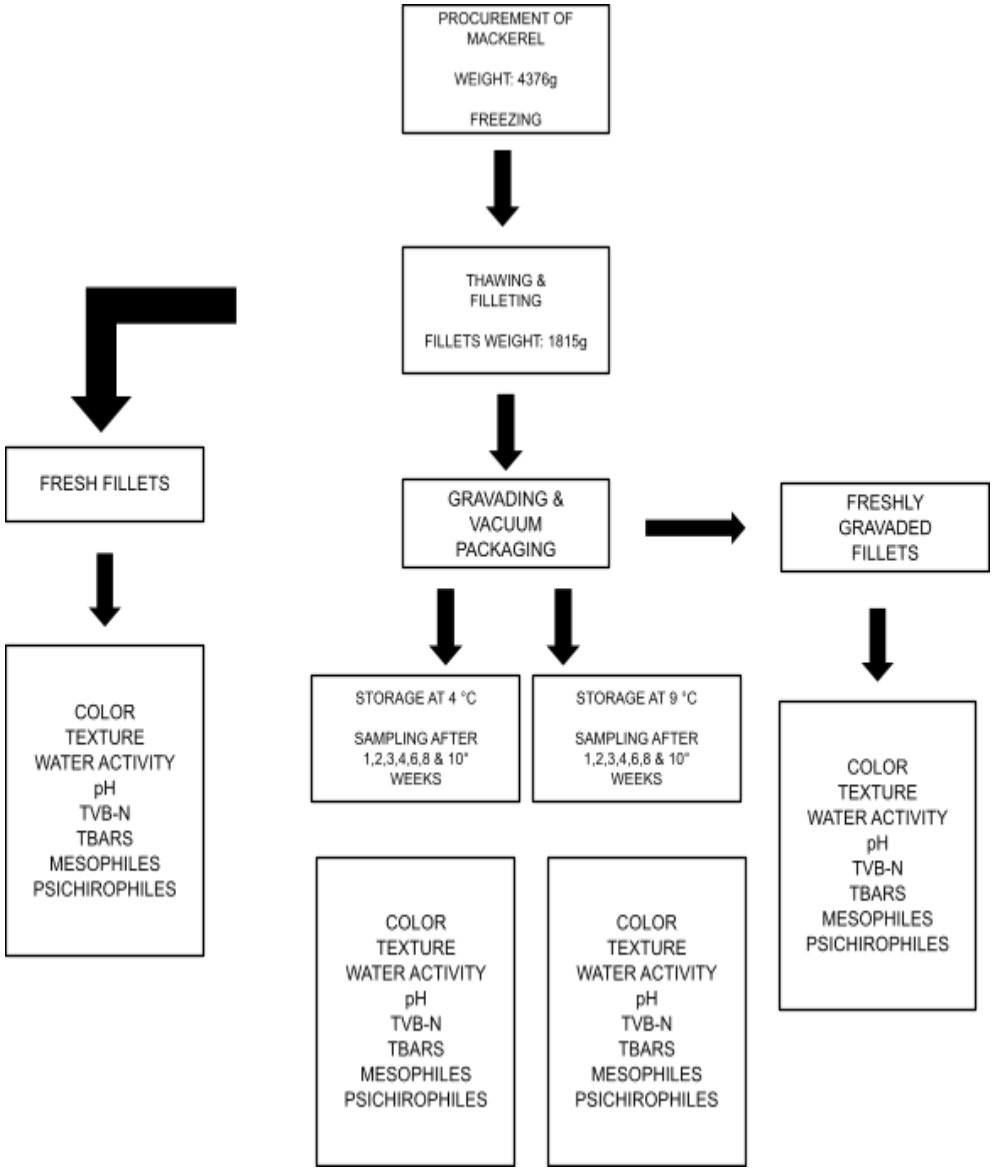

Figure 3.1 Flow diagram depicting the experimental design followed in this study.

3.2 RAW MATERIALS PROCESSING, PACKAGING AND SAMPLING

3.2.1 Weighing, freezing and storage.

A batch of mackerels *S. colias* (n=31) was bought from the Faro municipal market to the laboratory, wherein, chilled in ice, each specimen was weighed individually, and the batch was then weighed collectively (Figure 3.2). In preparation for the commencement of sampling, the batch was kept in a refrigerator at 3 ºC for < 2 h.

Figure 3.2 Weighing of the individual chilled mackerels in the Mackerel batch.

First, the fishes were placed side by side on trays before being frozen in an air-blast freezer at an airflow speed of 8.03 m/s at -25 °C. Temperature probes were used to monitor the freezing process over the course of an hour while the ambient temperature of the freezing chamber was also monitored (11:15 am to 12:15 pm). Readings were recorded until the fishes were judged to be completely frozen (Figure 3.3). The freezing curve representing this process is shown in Figure 3.4a. Afterwards, the already frozen batch (Figure 3.4b) was moved into freezing storage (-18 °C) where it was kept until the commencement of the experimental gravading and storage trial.

(a) (b)

Figure 3.3 Preparation and placement of the fishes in the air-blast freezer

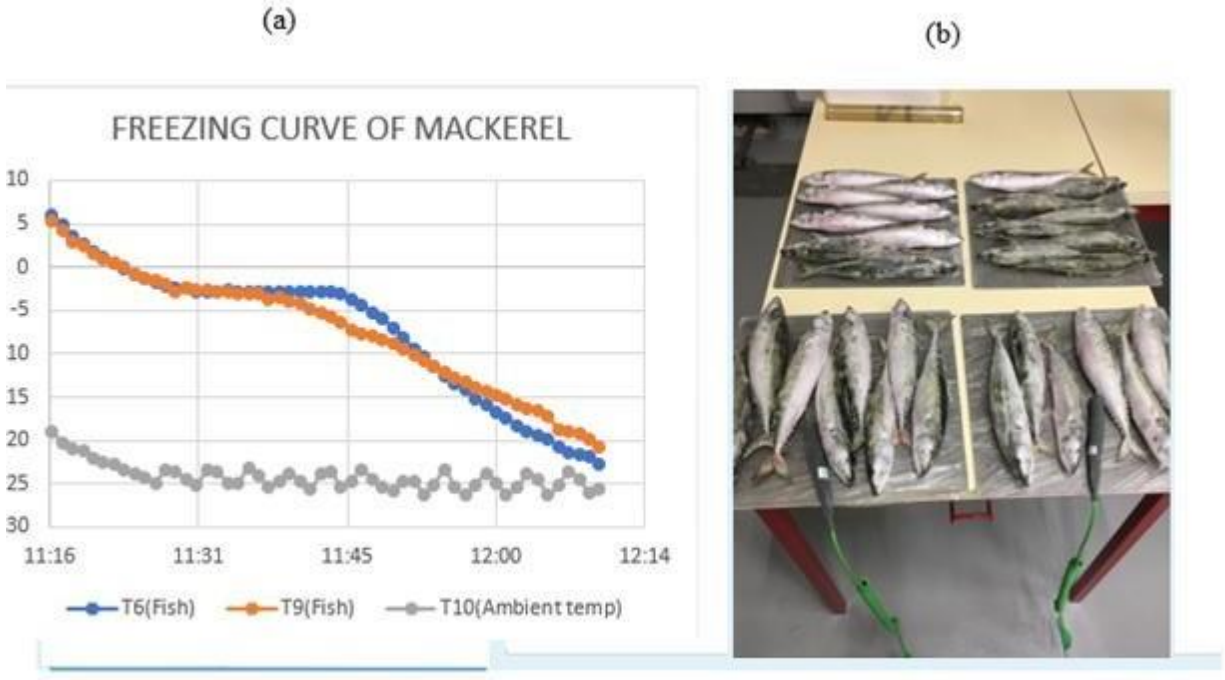

Figure 3.4 (a) Freezing curve of the process. (b) Frozen batch prior to storage in the freezer.

3.2.2 Thawing, Filleting, and Weighing.

Before the storage trial, the frozen batch was removed from frozen storage and moved into a refrigerator for natural thawing in controlled condition for 24 h. After thawing, each fish was weighed before filleting (W_{Fish}, g). Fishes were 140,5 ± 27,9 g. Filleting was manually done with a filleting knife under hygienic conditions and the fillets (Figure 3.5) were then weighed ($W_{Fillets}$, g. Fillets were, on average, 31,5 ± 7,1 g in weight. To determine the filleting yield (Yield, %) the following equation was used:

$$Yield = \frac{W_{Fillets}}{W_{Fish}} \times 100\% \qquad 3.1$$

The filleting yield (%) was 45,3% ± 7.1%.

Figure 3.5 Filleting and weighing.

3.2.3 Gravading

This process was carried out following closely Durmuş et al. (2017) and Rzepka et al. (2013). The process of gravading involved rubbing both sides of fillets with the gravad mix (Figure 3.6a), a previously prepared mixture of salt and sugar at a ratio of 1:2, at a ratio of 450 g of gravad mix to 1 kg of fillets. After application of the gravad, pairs of fillets were placed meat side to meat side and arranged in plastic trays (Figure 3.6b). A 1 kg/m^2 pressure was applied. The ripening – or gravading – was carried out for 48 h in the refrigerator (at 3 °C). After the first 24 h, each pair of fillets were flipped upside down in the trays. After 48 h ripening (or gravading), three fillets were sampled to be evaluated for quality parameters. The remaining gravaded fillets were vacuum packed (Figure 3.7b).

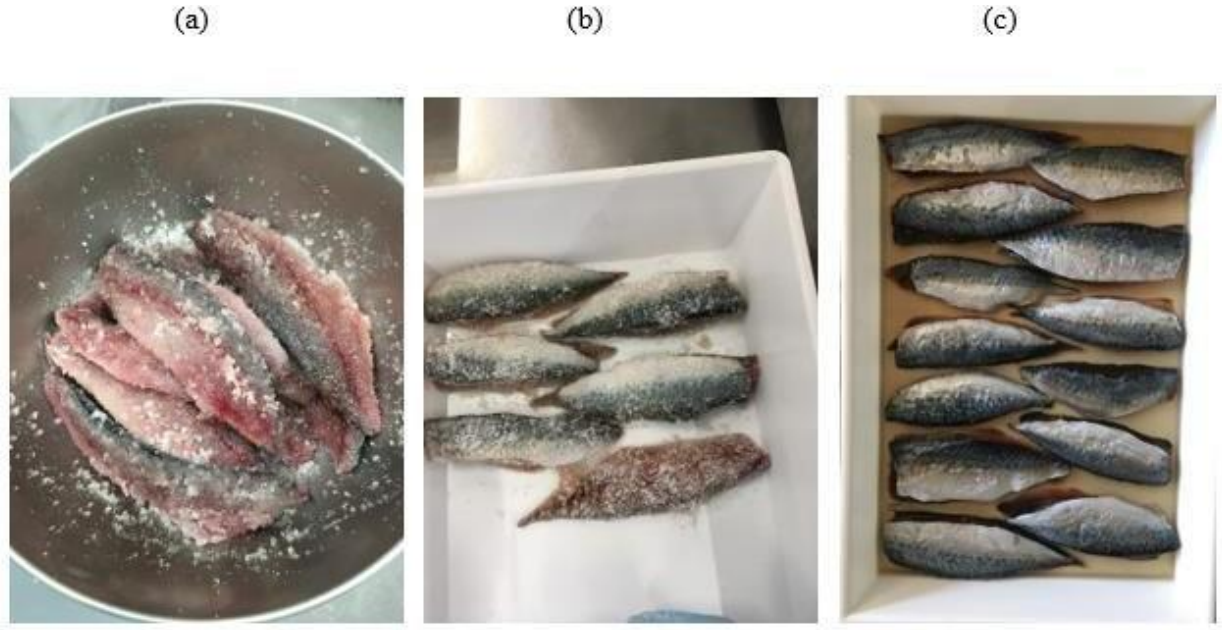

Figure 3.6 Gravading and ripening (a) application of the gravad mix to the fillets; (b) placing the fillets meatside to meatside after application (c) freshly gravaded fillets after ripening (48 h)

3.2.4 Packaging and Storage

The gravaded fillets were vacuum packed into airtight plastic bags, four fillets per pack (Figure 3.7) into Combitherm® XX (Wolff Walsrode AG, Germany) bags (200×200 mm) under vacuum (VP, at ca. 380 mm Hg). The plastic bag has an interior layer containing ethylene vinyl alcohol, an exterior layer of polyamide and a sealing layer of polyethylene. The packs were randomly divided into two groups, that were stored in the refrigerator at 4 °C and 9 °C, respectively, during the storage trial.

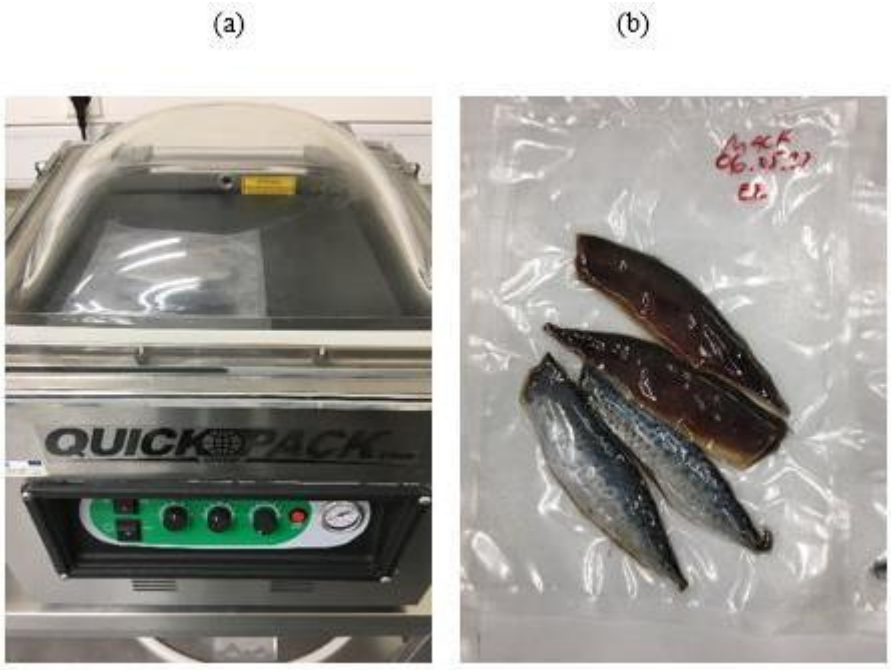

Figure 3.7 Vacuum packaging (a) The process (b) One of the final packages

3.3 ANALYSES

3.3.1 Color Parameters

3.3.2 Texture

For our study, a texturometer (LFRA Texture Analyzer, Brookfield Engineering Labs Inc., USA) with a 12.7 mm-diameter stainless steel spherical probe, which approached the sample at 1 mm s^{-1} and compressed 5 mm into the fillet, was used. TexturePro Lite v1.1 software (Brookfield Engineering Labs Inc., USA) was used to analyze the measurements (in kgf, where 1 kgf = 9.806 N). The peak load of the first compression cycle was used to calculate hardness (in N).

3.3.3 pH

In this study the pH was measured directly in the flesh of the fish fillets using a digital pH meter (model GLP 21, Crison, Spain) that was calibrated on a regular basis using standard solutions with pH 4 and 7 as per the manufacturer's recommendations. The probe for the semi-solid media (pH 2-11, 0-80 ^{o}C) was inserted into the muscle side of the fillets and the reading taken after usually 2 seconds. Three measurements per fillet taken and averaged, representing that fillet's pH value.

3.3.4 Water activity (a_w)

Herein, the water activity (a_w) of samples was determined directly using a water activity meter (Hygrolab, Rotronic Instruments, UK.). In short, 2 g of muscle was finely minced and evenly placed in the reading cells. The measured a_w values are in the range of 0—1 (or 0—100%).

After, the moisture/water content (%H) was obtained after drying the above-mentioned samples at 105 °C for >72 h (to constant weight) (cf. NP 151:1988). The %H is obtained from the difference between initial (W_i) and final (W_f) weight of samples as follows:

$$\%H = \frac{W_i - W_f}{W_i} \times 100\% \qquad 3.2$$

3.3.5 Thiobarbituric acid reactive substances (TBARS)

The TBARS content was determined by the direct extraction method described above, using a modified procedure of Norma Portuguesa NP-3356 (IPQ, 1990). Firstly, fish muscle (2 g) was minced and placed in a 50 ml centrifuge tube. 100 µl of butylated hydroxytoluene (BHT) (Riedel-de Haen, Germany) and 25 ml of trichloroacetic acid (TCA) (Scharlau, Germany) were measured and added to it. The mixture was then homogenized for 2 min with an Ultra-Turrax (model T25, Janke & Kunkel IKA Labortechnik, Germany) machine and filtered. An aliquot (5 ml) of the filtrate obtained was placed into a test tube and 5 ml of thiobarbituric acid (TBA) (Merck, Germany) reactive solution was added. The tube was placed in boiling water for 40 min. After, the tube was removed from the boiling water, left to cool to room temperature and the absorbance was read at 532 nm in a spectrophotometer (U-2000, Hitachi, Japan). This was repeated for each sample.

To prepare the TBARS standard curve, the malondialdehyde (MDA) stock solution was prepared by adding by adding 50 µl of Triethyl phosphate solution (TEP) (Merck, Germany) and 50 ml of 0.1N Hydrochloric acid (HCl) (VWR, United States) to a 50 ml Erlenmeyer flask. The flask was immersed in boiling water for 10 minutes and then left in cold water bath to cool to room temperature. An aliquot (2.4 ml) of the solution was transferred to a 100 ml volumetric flask and filled to volume with distilled water (this is the 0.1mM MDA stock solution). Then, 5 ml, 9 ml, 11 ml, 15 ml, and 20 ml of the stock solution was placed in separate 50 ml volumetric flasks and filled

to volume with distilled water. An aliquot (5 ml) from each of those solutions was placed in separate test tubes and 5 ml of TBA reactive solution was added to each of them. A blank was made by placing 5ml of distilled water in a test tube and adding 5ml of TBA reactive solution to it. All the test tubes were placed in a hot water bath (BTU-6, G. Vittadini, Italy) at 70- 80^0 C for 30 minutes. They were then moved into a cold-water bath, left to cool to room temperature and the absorbance read at 532 nm using a spectrophotometer (U-2000, Hitachi, Japan).

To calculate the TBARS value (mg MDA/kg fish), the following equation was used:

$$TBARS = \frac{72 \cdot MDA}{(m \cdot 5)(25 + m * H)} \qquad 3.3$$

where MDA is the concentration of MDA in the samples' solutions, m is the mass of sampled muscle (g) and H is the moisture content (%) of the fish muscle.

3.3.6 Total Volatile Base Nitrogen (TBV-N)

The content in total volatile basic nitrogen (TVB-N) was determined according to a modified version of the micro diffusion method by Conway and Byrne (1933). 10 g of sample was weighed to a centrifuge tube, 20 ml TCA (Scharlau, Germany) was added, and the mixture was homogenized using the Ultra-Turrax machine (model T25, Janke & Kunkel IKA Labortechnik, Germany) for around 2 minutes. The extract was filtered into a 50 ml beaker. The procedure was repeated for every sample.

Silicon paste was applied as the sealing agent to the Conway cells used. First, 1 ml of boric acid (Merck, Germany) was pipetted into the inner ring of every cell and two drops of indicator was added. For the blank cells, 1.5 ml of distilled water was pipetted to the outer ring before 1 ml of saturated potassium carbonate solution (Fischer, United States) was added. For the control cells,

0.5ml of distilled water, 1 ml of ammonium sulphate standard solution (AppliChem, Germany) and then 1 ml of saturated potassium carbonate solution (Fischer, United States) were pipetted to the outer ring in that order. In the outer ring of the sample cells, 0.5 ml of distilled water, 1 ml of the sample extract and finally, 1 ml of saturated potassium carbonate solution (Fischer, United States) were pipetted in that order. The addition of saturated potassium carbonate solution was the last step in every case and was done with the Conway cell units slanted with cover and closed immediately to avoid escape of volatile nitrogen on mixing.

The cells were rocked gently to mix and then placed in the oven at 40 °C for 90 minutes. Afterwards, the inner rings were titrated with HCl (VWR, United States) until a persistent pinkish coloration develops. To calculate the TBV-N value (mg N/100 g), the following equation was used.

$$\text{TVB-N} = \frac{21 \times (V2 - V0)}{(V1 - V0) \times V3 \times m \times (20 - H)} \quad 3.4$$

where V0 is volume of HCl used in titrating the blank, V1 is the volume of HCl used when titrating the control, V2 is the volume of HCl used to titrate the sample, V3 is the volume of sample used (1 ml), m is sample weight and H is the moisture content of the sample.

3.3.7 Total Aerobic and Psychrophilic Bacterial Counts

First, fish dilution solution was made by placing 10 g of chub mackerel fish muscle into sterilized Stomacher® bags containing 90 mL of peptone water with sodium chloride (Merck, Germany) and homogenized for 4 minutes using a Stomacher® (Stomacher 400, Seward Ltd., UK). Serial dilutions were made which increased throughout the experiment, wherein 1 mL of each suspension was transferred to a tube and 9 mL and diluted. Before each inoculation, the tube with the sample was homogenized in a Vortex mixer (VWR Analog Vortex Mixer, United States), where all tubes, whenever opened and before being capped, were flamed to prevent possible contamination risks. Dilutions were always carried out using this methodology during the analysis. The inoculation method recommended by the standard (NMKL, 2006) is surface spreading, which consists of inoculating 0.1 mL of each dilution in the respective petri dish containing the Long and Hammer (L&H) medium, spreading the inoculum evenly across its surface, with the aid of a sterile spreader. Incubation was carried out with the plates inverted at a temperature of 15 °C ± 1 °C for 5-7 days for aerobic bacteria and 6 °C ± 1 °C for 5-7 days for the psychrophilic bacteria.

All plates were analyzed visually to perform microorganism counts, checking for typical colonies associated with each culture medium. The microbiological results obtained, i.e., number of colony forming units per mass unit (CFU/g) were log-transformed, log(CFU/g).

3.3.8. Data analysis

All results are presented as comparative charts (column and line charts), including standard deviation (±SD) as a measure of variation. ANOVA (two-way) was used to test differences in the quality parameters assessed among sampling occasions (factor stages) and between the two storage temperatures assayed (factor temperature). The post-hoc multiple comparisons method of Tukey HSD was carried out to clarify eventual significant differences (Esteves, 2010).

4.0 RESULTS AND DISCUSSION

4.1 COLOR PARAMETERS.

Figure 4.1 shows the plot for the lightness (L*). Changes in L* was significant (ANOVA, $p<0.05$; Table 3.1). Highest values of lightness were recorded for the fresh mackerel fillets (39.43 ± 3.93), which decreased on gravading (29.52 ±2.41). The lightness improved from that point on, after two weeks of storage for the gravaded fillets stored at 9 ^{o}C and after three weeks of storage for the 4 ^{o}C samples. There were some increases and decreases along the way for lightness, but the lightness parameters never reached the peak attained by the fresh samples for lightness. The consistent zigzag change was visible in both the stored samples, starting to increase in the second week of storage to decrease in the following week and continuing in that pattern. The 4 ^{o}C samples showed the higher lightness values of the stored samples by the end of the experiment (35.46 ±0.36) (Tukey HSD, $p<0.05$).

The L* value decreased in both studied temperature with storage. This is expected as the fillets lost the luminosity of freshness and turned shiny but dark after gravading. These changes could also be associated with a dehydration process due to the variations in temperature at each sampling point after opening the refrigerated chamber (Paola & Isabel, 2014). Hamre et al. (2003) found the same results in their herring studies (*Clupea harengus L.*).

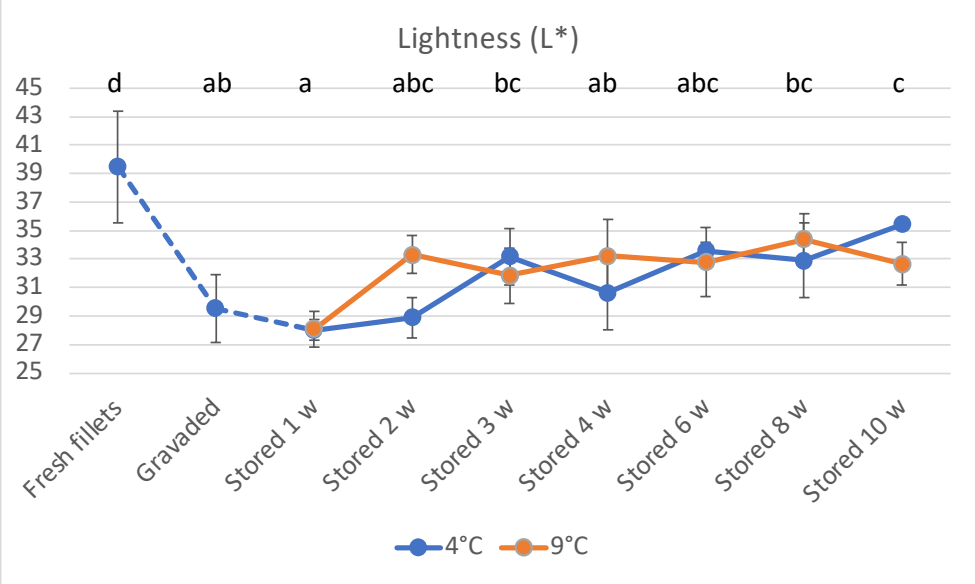

Figure 4.1 Color measurements; L (lightness, black = 0, white =100) according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Each point represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)*

Table 4.1 Results of two-way ANOVAs per quality parameter considering factors Stage (fresh, gravaded, and times 1 to 10 weeks of storage) and Temperature (4°C and 9°C).

Parameters	Factors	F	p-value
L*	Stage	12,75	**<0,001**
	Temperature	0,45	0,506
	Stage * Temperature	1,42	0,220
a*	Stage	2,25	**0,047**
	Temperature	0,41	0,527
	Stage * Temperature	1,44	0,215
b*	Stage	11,25	**<0,001**
	Temperature	2,40	0,130
	Stage * Temperature	0,43	0,896
C	Stage	6,54	**<0,001**
	Temperature	1,10	0,301
	Stage * Temperature	0,45	0,880
h°	Stage	4,77	**<0,001**
	Temperature	0,08	0,781
	Stage * Temperature	0,95	0,488
Hardness	Stage	12,78	**<0,001**
	Temperature	0,06	0,815
	Stage * Temperature	2,13	0,059
pH	Stage	39,01	**<0,001**
	Temperature	0,97	0,332
	Stage * Temperature	0,92	0,508
Moisture content	Stage	36,58	**<0,001**
	Temperature	4,37	**0,044**
	Stage * Temperature	1,91	0,091
aW	Stage	84,87	**<0,001**
	Temperature	15,72	**<0,001**
	Stage * Temperature	7,04	**<0,001**
TVB-N	Stage	35,56	**<0,001**
	Temperature	32,09	**<0,001**
	Stage * Temperature	3,32	**0,007**
TBARS	Stage	14,51	**<0,001**
	Temperature	8,05	**0,008**
	Stage * Temperature	1,98	0,082
Total Aerobic Bacteria	Stage	15,20	**<0,001**
	Temperature	2,15	0,153
	Stage * Temperature	2,10	0,075
Total Psychrotrophic Bacteria	Stage	9,07	**<0,001**
	Temperature	0,00	0,967
	Stage * Temperature	1,09	0,395

In figure 4.2, the redness/greenness parameters are shown. The fresh and gravaded fillets started off as greenish (a* < 0) but after a week of storage, 4 ºC filets show a little increase in redness which was short-lived and prove to be the case for most of the experiment. The highest value for redness came after 4 weeks of storage at 4 ºC (1.28 ± 2.04). There was no particular pattern shown in this parameter over the course of the experiment, but changes among sampling/processing changes were significant (ANOVA and Tukey HSD, $p < 0.05$).

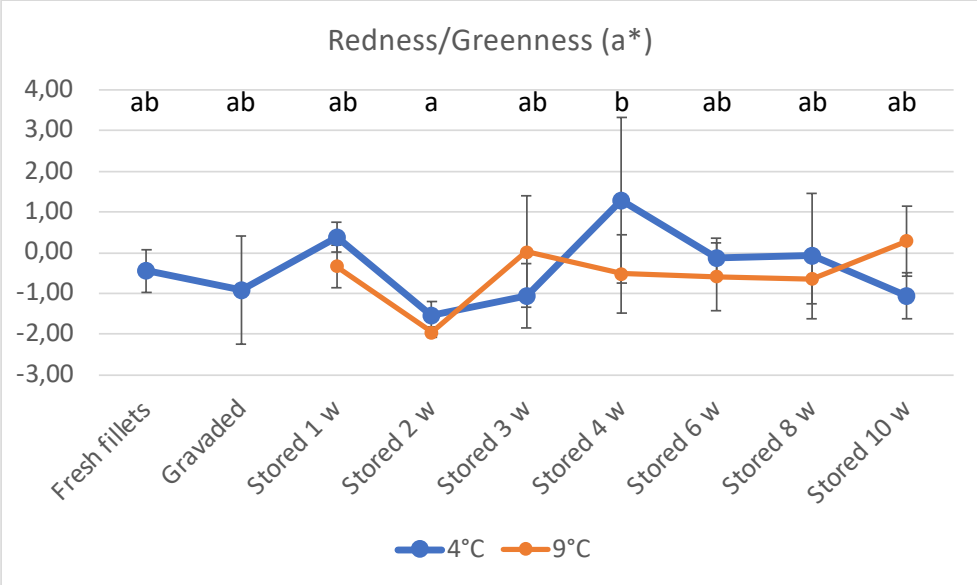

Figure 4.2 Color measurements; a - (Redness/greenness (a*) (Red > 0, Green <0)) (mean ± SD), according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Each point represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)*

There was a significant drop (Tukey HSD, $p < 0.05$) in yellowness from the fresh fillets to the gravaded fillets (Figure 4.3). There was a slight increase of b* this after 1 week of storage at 4 ºC, which was followed by another decline. From the third week of storage, b* continued to increase consistently until the end of the experiment. For the gravaded fillets stored at 9 ºC, there was also a consistent rise in this parameter from the third week, barring a drop after week 4. The progression was consistent after then, culminating in the highest value of the parameter b*, recorded after the final week (5.64 ± 0.64), toppling the initial high of 5.55 ± 1.30 recorded by the fresh fillets.

The b* tendency to the yellow color as storage progresses could be linked to the oxidation process

during storage, which leads to formation of colored compounds, known as Schiff bases (Wetterskog & Undeland, 2004). The Schiff base are formed through the reaction between aldehydes that are produced during lipid oxidation and protein amino groups, which causes the protein to lose its functional properties (Chopin et al., 2007). This relationship between yellowing and non-enzymatic browning reactions was examined by Tongnuanchan et al. (2011) in films made from tilapia (*Oreochromis niloticus*) muscle protein, associating higher TBARS values with high values of b*.

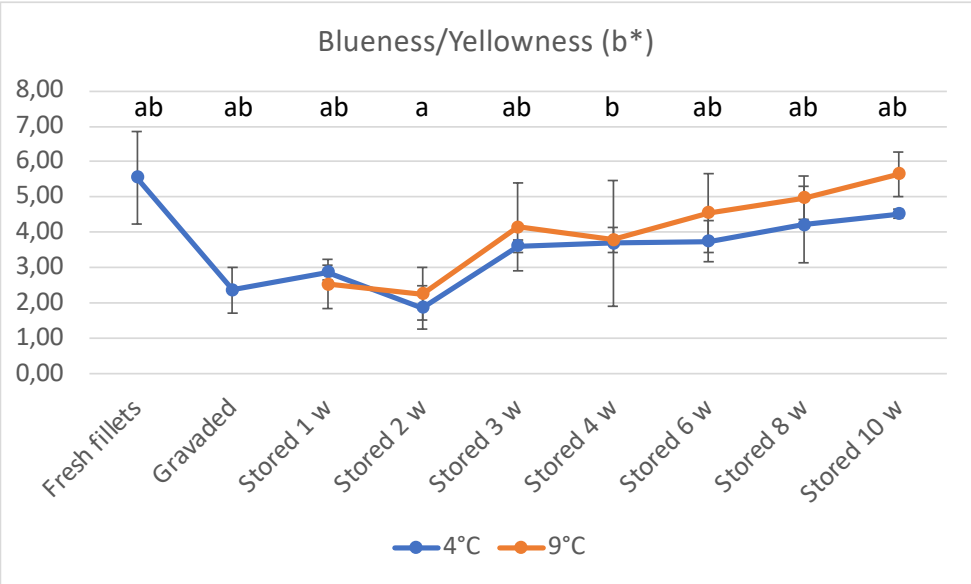

Figure 4.3 Color measurements; b - (Blueness/Yellowness) (mean ± SD), according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Each point represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)*

After a significant drop (ANOVA and Tukey HSD, $p < 0.05$) in the chroma (C*) value from the fresh fillets to the gravaded fillets, both stored gravaded samples at 4 ^{o}C and 9 ^{o}C experienced consistent rises, barring a brief drop in week 4 for the 9 ^{o}C and week 6 for the 4 ^{o}C samples (Figure 4.4). The highest value for chroma was recorded in the last week of experiment by the 9 ^{o}C samples (5.79 ±0.63), eclipsing the previous high by the fresh samples (5.75 ±1.22).

In Figure 4.5, the hue angle (h°) values varied significantly (ANOVA and Tukey HSD, $p < 0.05$) among stages of sampling/processing but show no trend except the highest value being recorded after two weeks of storage for both of the stored samples. This rise followed a massive decline in

hue angle values recorded from the freshly gravaded fillets and the stored gravaded fillets after 1 week for both temperatures.

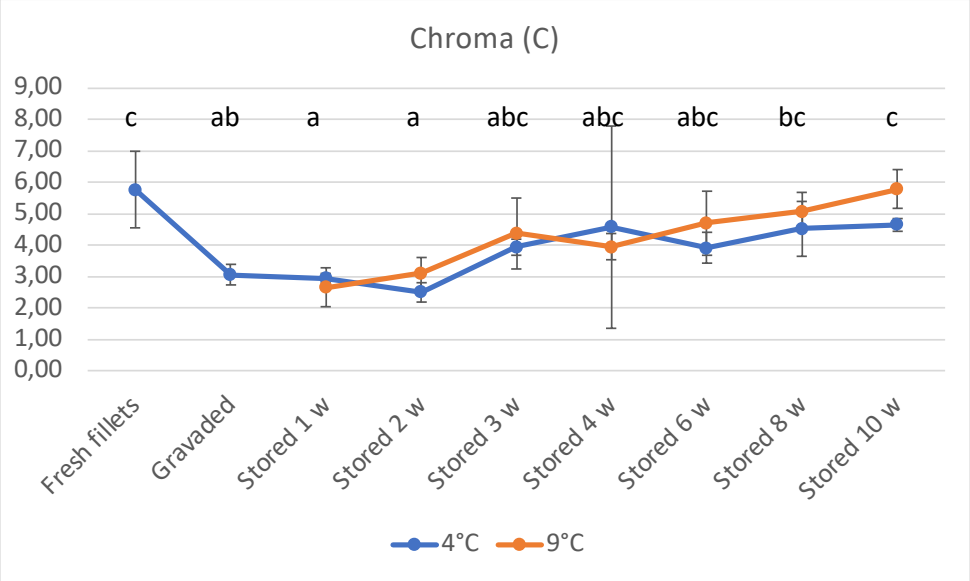

Figure 4.4 Color measurements; C - (Chroma) (mean ± SD), according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Each point represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)*

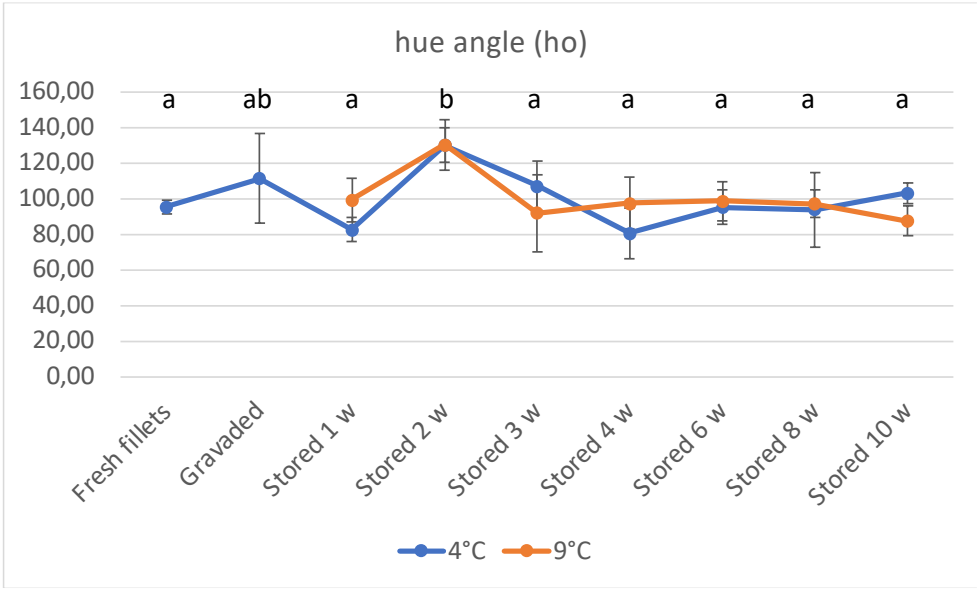

Figure 4.5 Color measurements; ho - (hue angle) (mean ± SD) according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Each point represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)

4.2 TEXTURE

Texture was measured as the peak load required to cause a deformation to the surface of the fillets. The results are shown on the plot in Figure 4.6. There were significant differences among stages of processing (ANOVA and Tukey HSD, $p<0.05$) but not between temperatures tested. Unsurprisingly, the lowest peak load recorded was for the fresh fillets. There was consistent increase from this point up until week 4 of storage when the highest peak load was recorded for the 4 ^{o}C stored fillets (861.53 ± 233.70 gf). There was no pattern to how the peak load varied between the 4 ^{o}C fillets and the 9 ^{o}C fillets (ANOVA, $p > 0.05$).

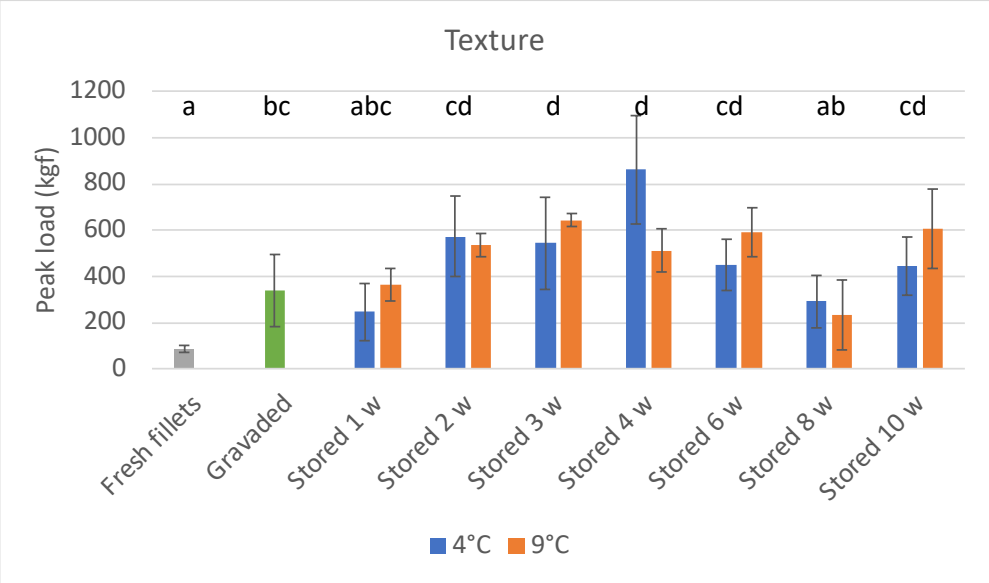

Figure 4.6 Maximum peak load required to cause a surface deformation to the fillets (mean ± SD). Each column represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)

Hardness is an important aspect of fish texture analysis. Textural properties of our raw fillets, freshly prepared and gravad shows increase in the peak load. This implies progressive hardness. This agrees with Durmus et al. (2017) that claims that the process of gravading causes increased hardness of fillets. The principal component of fish muscle protein is the skeletal muscle formation combined with other materials such as water and fat. Protein is important. The textural parameters of the fish muscle are therefore related to protein and water content (Durmus et al., 2017). Since gravading is in essence an osmotic dehydration process that removes water, hardness was expected to increase, particularly between fresh and gravaded fillets.

4.3 pH

The initial pH of the fresh samples was recorded as 5.74 ± 0.05, which decreased to 5.60 ± 0.03 with the gravaded fillets (Figure 4.7). There were significant differences among stages of processing (ANOVA and Tukey HSD, $p < 0.05$). There was a general increase up from that point, reaching peak level after 4 weeks of storage (6.07 ± 0.10 for 4 ^{o}C samples) and then decreasing from then till the final week of sampling. Often, the 4 ^{o}C samples recorded higher pH values than the 9 ^{o}C samples, but differences were not statistically significant (ANOVA, $p > 0.05$).

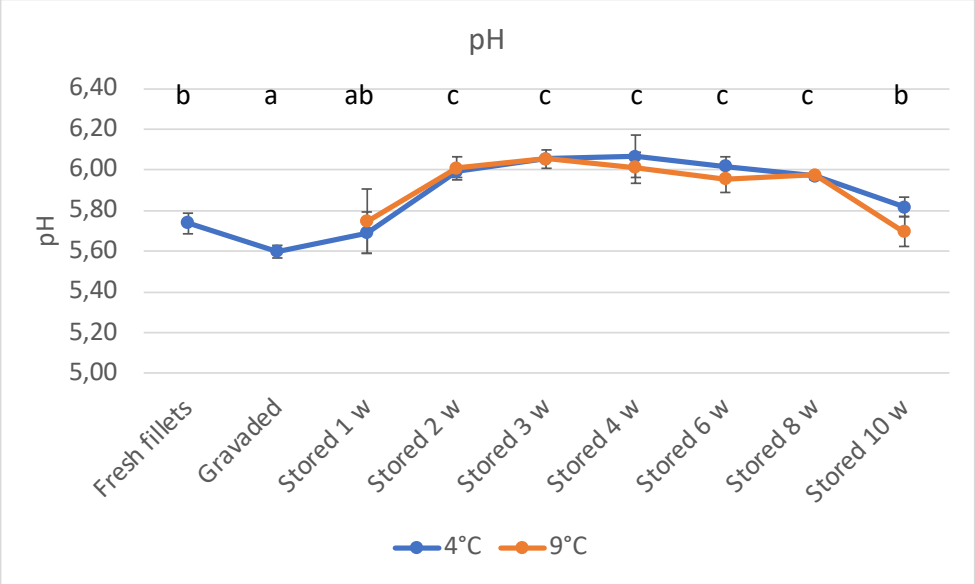

Figure 4.7 pH (mean ± SD). Each column represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)

Measuring pH of fish muscles gives can give an indication of the production of alkaline metabolites caused by spoilage bacteria. Fish pH after mortem varies from 6 to 7.1 based on species, fishing method, fighting diet of fish harvest and other factors fish harvesting hard (Li et al., 2011). pH over 7.1 could be considered a spoilage index of fish (Ozyurt et al., 2009). The pH variation of our gravads as shown in Figure 4.7 is less than that value all through this experiment which would have meant our gravad never spoiled. This may not be the case as the spoilage TVN-N values and oxidation status TBARS values were already high by the fourth week of storage. Seemingly, pH is not a reliable quality indicator as the variation does not show a lot. Not even the difference in temperature seem to make a lot of difference, which is why TVB-N was paid more

attention to.

4.4 WATER ACTIVITY

As expected, the water activity is highest in fresh mackerel (0.985 ±0.003). There was a sharp decrease for the freshly gravaded fillets (Figure 4.8). Differences among processing stages were significant (ANOVA, $p < 0.05$). The storage of gravaded fillets at 4 ºC showed a significant rise after six weeks storage and then decreased down to 0.860 ± 0.013. The lowest value was recorded for the 9 ºC samples after the last week of experiments with 0.809 ± 0.006. In fact, a significant interaction effect was found (ANOVA, $p < 0.05$) between factors stage of processing and storage temperature.

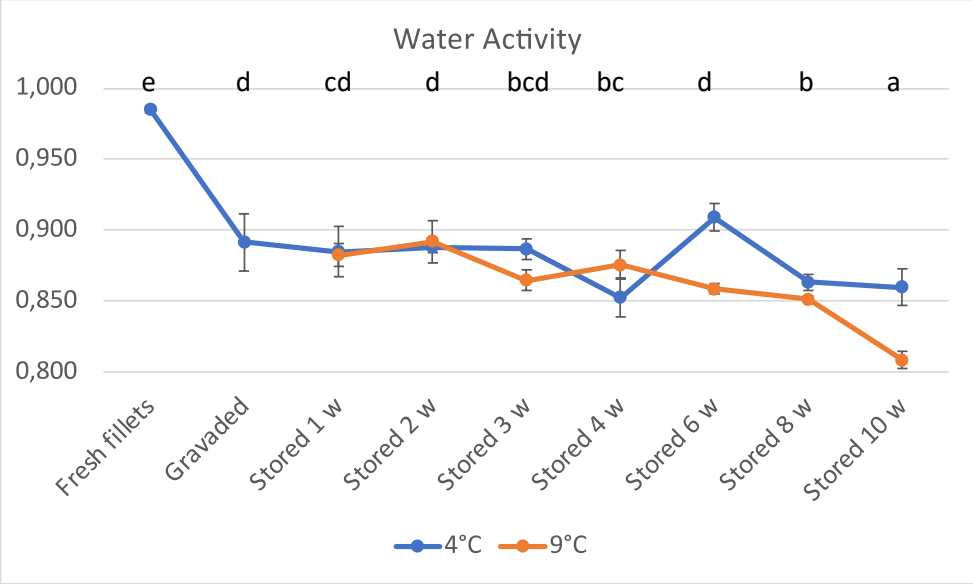

Figure 4.8 Water activity (mean ± SD). Each point represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)

The gradual decline can be expected as over the weeks, the fillets were harder, indicating water drip loss or hardness. The progressive decrease in water activity may also be due to salts and sugar saturation of the fillet and the release of salt during maturation (Surowka et al., 2021). When certain substances such as salt and sugar are dissolved in water, water activity decreases, and water are less accessible to microorganisms, in other words it will be blocked (Nemeyer et al., 1997). This results in a “harsher” environment for microorganisms. At values of water activity of 0.80 or below, only yeasts and molds and halophitic bacteria can strive (Hall, 2011).

This reduction can also indicate proteins denaturation because the capacity of maintenance of active water is directly related to protein myofibrils linked (Suvanich et al., 2003).

4.5 TVB-N

The total volatile base nitrogen decreased slightly after gravading (Figure 4.9). A marked increase was shown from then on for the stored samples. The samples stored at 9 ^{o}C recorded higher TVB-N values than the samples at 4 ^{o}C, barring after the first week of storage when the samples at 4 ^{o}C recorded a marginally higher TVB-N values. The highest TVB-N value was determined in the samples stored at 9 ^{o}C in the last week of experiment signaling the end. These patterns were reflected in a significant interaction effect (ANOVA, $p < 0.05$) between factors stage of processing and storage temperature.

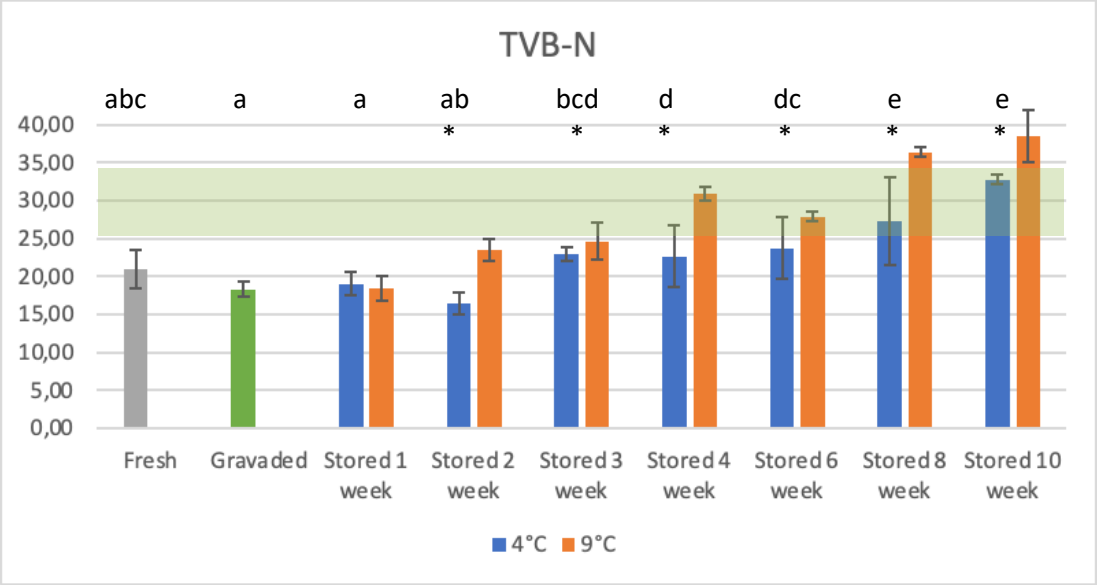

Figure 4.9 TVB-N (mean ± SD) in TVB-N ABVT mg N/100g. Each column represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05) Asterisks denote significant differences between temperatures at stage following significant interaction effects (ANOVA, p<0.05)

The total volatile bases accumulated in spoilage of fish include ammonia, methylamine, dimethylamine, and trimethylamine, produced by the degradation of protein and nonprotein components of fish meat from spoilage bacteria (Liu et al., 2013). For fresh fish, the upper threshold of TVB-N is 30 mg/100 g (Bensid et al., 2014; Duman & Ozpolat, 2015). For certain

species of fishes, the European commission has set the maximum acceptable TVB-N limit ranging from 25 mg to 35 mg/100 g (EC, 2008). This could mean our product started to enter unacceptable levels after 4 weeks of storage, definitely after 8 weeks. The TVB-N values in our study increased slowly up till this point in samples stored in both 4 ^{o}C and 9 ^{o}C, and increased sporadically after seemingly agreeing with these limits, as the spoilage got more rapid at this point. We generally recorded higher TVB-N values for the 9 ^{o}C samples all through the study. This agrees with Yassoralipour et al. (2013) that recorded significantly higher TVB-N levels in Barramundi stored at 8°C than those kept at 0°C. Volatile bases are mostly generated by endogenous and microbial enzymes and it is known that their activities are greatly influenced by temperature. Microbial activity increases with increase in temperature and can explain the higher level of TVB-N formation in gravads stored at 9 ^{o}C. In contrast, Fans et al. (2014) reported lower TVB-N values in sugar–salted black carp fillet than salted and control fillet and credited their findings to the production of organic acid in samples with added sugar for volatile base neutralization. Seemingly, this was not the case in our study.

4.6 TBARS

Figure 4.10 presents the values of the TBARS measured in fresh, gravaded and stored gravaded fillets at 4^{o}C and 9 ^{o}C. The initial TBARS value of the fresh fillets was 15.52 ± 4.77 mg MDA/kg. The TBARS values rose for the freshly gravaded fillets and showed marked consistent increase for all the stored sample, barring a brief decrease after 1 week of storage and the visible decrease in the last week of experiment (significant factor stage of processing, ANOVA $p < 0.05$), which could be due to the low number of samples used for the last experiment (four fillets were sampled instead of six). The 9 ^{o}C samples recorded consistent and significant (ANOVA, $p < 0.05$) higher TBARS values than the 4 ^{o}C samples, except after the first week of storage.

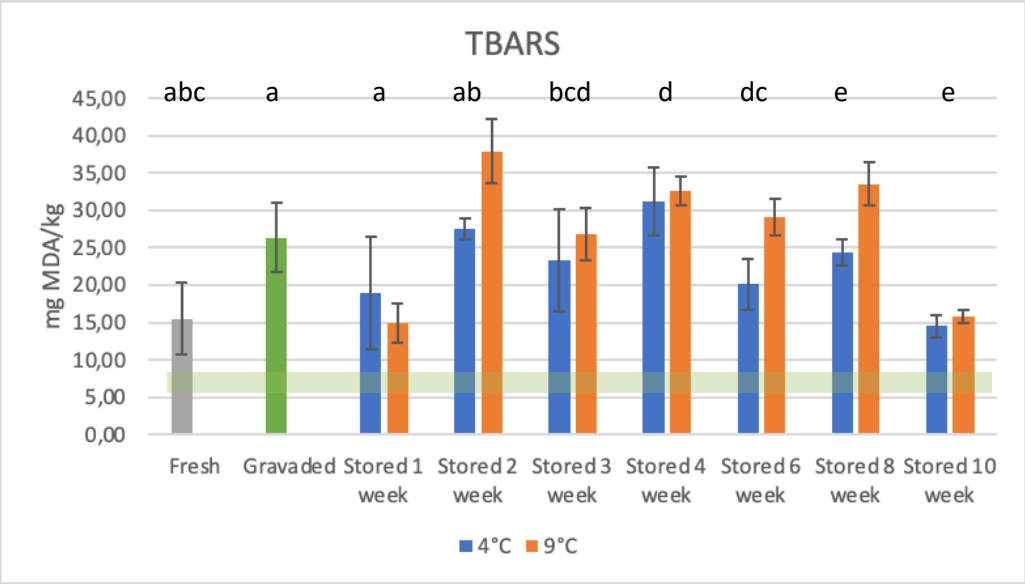

Figure 4.10 TBARS (mean ± SD) in mg MDA/kg. Each column represents an average of three samples. Distinct letters indicate significant diffrences (Tukey HSD, p<0.05)

TBARS value was used to examine the progression fat deterioration and it was observed that the value increased after gravading. The increase in the TBARS value from the fresh fillets to the gravaded fillets might be due to the characteristic that the process of gravading enhances muscle dehydration (Michalczyk et al., 2008). For most of our experiment on the stored gravads, it could be seen that the 9 ^{o}C stored sample showed higher TBARS values as the storage time increases. This agrees with the work of Dawood et al. (1986) on trout, that pointed out that the higher the temperature and the longer the time, the higher the TBARS values recorded.

The value of TBARS increased until week 10 when it experienced a sharp decline to close off the experiment. This decrease might relate to the interaction of MDA with other components like decomposition of proteins. Therefore, this parameter alone is not reliable for the measure of actual lipid oxidation (Rzepka et al., 2013).

4.7 TOTAL AEROBIC AND PSYCHOPHILIC BACTERIAL COUNTS

In terms of the microbiological parameters, the total aerobic count was highest in the fresh samples (4.06 ± 3.60 log(CFU/g)) (Figure 4.11). This abundance decreased steadily, with exception of week 4 and 8. There was no pattern observed between the 4 ^{o}C stored fillets as each took turns

posting the highest values. The 9 ^{o}C samples had the highest count (3.59 ± 3.65) among the stored samples by the end of sampling, but still did not reach the count of the fresh samples. There were significant differences among stages of processing (ANOVA and Tukey HSD, $p < 0.05$) but not between storage temperature (ANOVA, $p > 0.05$).

Like in the total aerobic counts, the psychotropic bacterial counts were highest for the fresh samples (2.79 ± 2.50 log(CFU/g)) (Figure 4.12). There was a steady decrease until the second week of storage when the values started to marginally increase again. There were no counts recorded after 8 weeks of storage for the total psychrotrophic bacteria. There was also no steady pattern between the stored fillets with both temperatures posting higher counts in different weeks. The 9 ^{o}C samples had higher counts (2.18 ±0.00) than the 4 ^{o}C samples (2.00 ± 1.70) by week 6 of storage, which turned out to be the final week with psychotropic counts. Again, there were significant differences among stages of processing (ANOVA and Tukey HSD, $p < 0.05$) but differences between storage temperature were not significant (ANOVA, $p > 0.05$).

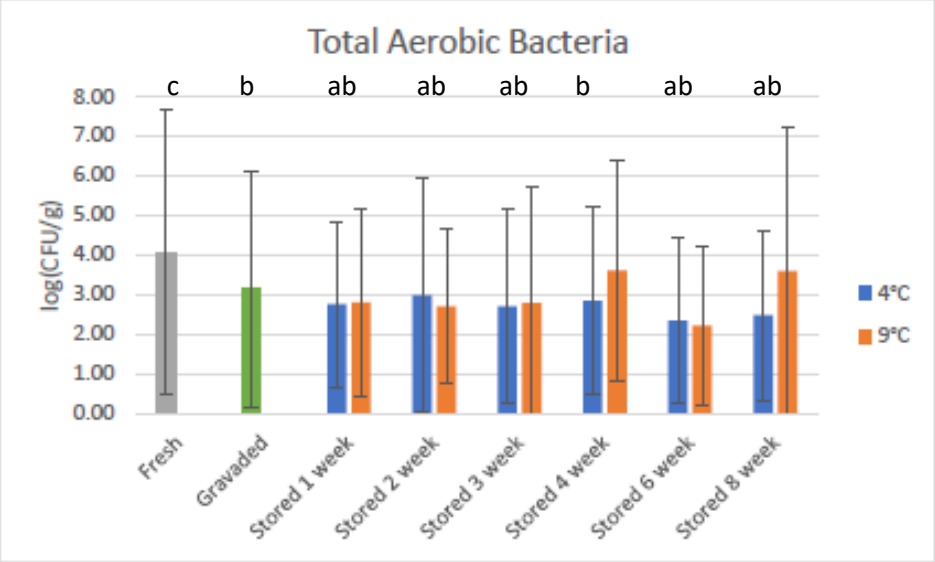

Figure 4.11 Total mesophilic counts (mean ± SD) in log (CFU/g). Each column represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)

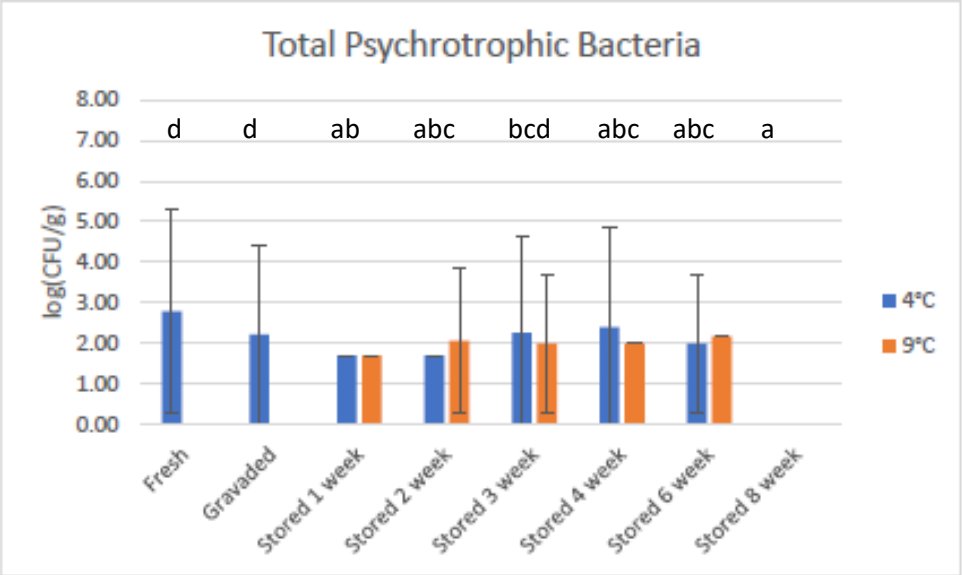

Figure 4.12 Total psychotropic counts (mean ± SD) in log (CFU/g). Each column represents an average of three samples. Distinct letters indicate significant differences (Tukey HSD, p<0.05)

The highest value for total aerobic count was around 4.06 log(CFU/g). This level is common for many fish species (Ozogul et al., 2008) and not exceeding this level even after gravading shows the high quality of both our gravad and the raw material. This is around the same range with both the microbiological counts level of 5.1 log (CFU/g recorded by Rzepka et al. (2013) for Atlantic bonito gravads and 4.62 log(CFU/g) for Durmus et al. (2017) on carp gravads.

IFST (1999), after only eight days of storage at 8°C, reported more rapid growth in total bacteria, particularly for cold smoked salmon that exceeded spoilage at a level of 7 log(CFU/g). This is the proposed conventional limit of 7 log (CFU/g) for foods by the International Commission on the Microbiological Specifications for Foods (ICMSF, 1986). Lyhs et al. (2001) believe that the decisive impact on gravads is the high microbiological contamination. In their view, for products stored at 3 and 8 °C, the critical level is the number of bacteria that are equal to or greater than 6 and 7 log(CFU/g). In this study, gravading and vacuum packaging slowed bacterial growth during cold storage like also in the study of gravading of cold stored Atlantic bonito (*Sarda sarda*) by Rzepka et al. (2013), mainly due to the lack of oxygen in the packaging and the presence of natural conservation substances (sugar and salt).

5.0 CONCLUSION AND FINAL REMARKS

Partial dehydration and absorption of sugar and salt by the muscle tissue of the fish are the essence of the gravading process. This, in conjunction with natural post-mortem processes, leads to microstructural modification in fish, which can be modified to make a great product.

The color parameters did not show a particular trend over the course of the experiment, except for the lightness parameter L* which decreased as the time goes on probably due dehydration process due to the variations in temperature at each sampling point after opening the refrigerated chamber.

Water activity reduced sufficiently into the final weeks to around 0.80, a stage only yeasts and molds and halophytic bacteria can strive, portraying good raw material and product stability having harsh conditions for microorganisms.

The texture of the mackerel underwent major changes due to the increased sugar and salt that augments substantially the osmotic pressure and potentiates removal of the water from the fish tissues. This made the hardness parameter increase until the fourth week of storage after which it starts to decrease, potentially pointing to the notion that regardless of temperature, mackerel gravads may start to lose some quality after four weeks of storage. This is backed up by the TVB-N increasing above the 30 mg N/100g mark after week 4, and the TBARS values following suit after the same week. The last TBARS reading was low, but this is possibly because at this point, we did not have much raw material to work with as in the weeks prior and the reading may not be reliable.

In this work, the standardized 1:2 salt to sugar ratio that gained the best acceptance in Pankyamma et al. (2020) was used. The bacteria count was low and did not exceed the normal level of the fish species that have most popularly been gravaded, which means mackerel can make good gravads. Over time, there was a decrease in the water activity of our gravads (to a_W of ca. 0.8-0.86) and increased hardness pointing to the impact of sugar and salt presence, which will make water less accessible to microorganism and increase shelf life.

Combination of vacuum packaging with gravading slowed down bacterial growth during cold storage. Average counts of total aerobic and psychrophilic bacteria did not exceed 4 and 2 log (CFU/g), respectively, during the storage trial of 10 weeks.

Although combining vacuum packaging seem to keep the effect of spoilage microorganism at bay for this product, more work needs to be done to increase the shelf life of mackerel gravad as the quality parameters seem to tail off after four weeks of storage, regardless of the temperature. Moreover, assessing the organoleptic attributes of the product and their change through time and the willingness to consume this sort of products by consumers other than Scandinavians and Nordic is in order.

6.0 FUTURE WORK

Further study of the oxidation-indicative parameters (namely Peroxide Value and TBARS) is required to check suitability and representativeness of methods

Furthermore, efforts need to also be made to study other occurrences and changes in abundance of other microorganisms

Most importantly, studies on sensory quality and acceptability of the "product" to make it appealing to consumers outside of Scandinavian and Nordic countries should also be carried out.

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