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Storage and processing of mackerel - effect on lipid stability.

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Biotechnology
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Preface and acknowledgments

This study is a master thesis in 2 years international master program in Biotechnology at the Norwegian University of Life Science and Technology, NTNU. The research was performed at the biotechnology department at NTNU in collaboration with SINTEF Fisheries and Aquacultures as a part of the project ProHealthPelagic. The research was carried out from Fall-2016 to Spring-2018 semesters and counts for 60 credit points.

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Abstract

Atlantic mackerel (*Scomber scombrus*) provides numerous important nutrients linked to lipids, proteins and other bioactive compounds. Mackerel is a very good source of long chain n-3 polyunsaturated fatty acids which have a lot of beneficial health effects, for example on cardiovascular diseases. Unfortunately, due to the high amount of unsaturated fatty acids, mackerel is highly prone to lipid oxidation leading to a decrease of health benefits, while decreasing sensory (undesirable taste and flavor) and nutritional profile. In order to protect Atlantic mackerel from lipid oxidation during storage a number of preservation/mild processing methods were explored in this project.

The main purpose of the project was to prolong shelf-life and retain sensory quality and nutritional properties of the mackerel including preserving vitamins, minerals, proteins, lipids etc. The aim of this master thesis was to study the effect of different types of storage (chilling, superchilling and freezing) and duration of storage on the oxidation stability in mackerel lipids. The skin-on fillets were chilled for 0, 2, 5 and 7 days; superchilled for 2, 5, 9 and 14 days; and frozen for 2 days, 8 months and one year. Total lipid content, primary and secondary oxidation products were evaluated by assessing the peroxide value (PV) and TBARS (chloroform and water/methanol phase) respectively. Color parameters were measured in order to assess the lightness, redness and yellowness. Additionally, the effects of various sous-vide time-temperature regimes and their influence on lipid oxidation were evaluated during chilled storage. The fillets were cooked in sous-vide bath for 10, 15 and 20 minutes at 60°C, 75°C and 90°C and further stored for 1, 3 and 7 days on ice. Color parameters and formation/accumulation of products of lipid oxidation were measured.

The total lipid content was range between 19,80% and 22,68%. The PV values were well within the recommended limits. None of the samples exceeded 10 meq O_2/kg oil and the highest obtained PV was 6,43 meq O_2/kg oil for superchilled fish at day 5th. Superchilled samples were in general characterized by slightly higher PV than chilled and frozen. The results from evaluation of secondary oxidation products show low values of TBARS in chloroform phase (maximum TBARS for superchilled mackerel at day 5^{th} was 2,35 μmol TBARS/g lipid), which varies depending on type of storage. TBARS for methanol/water phase slightly decreased during chilled storage, increased during freezing and for superchilled samples the level of TBARS was rather stable. Color measurements revealed a correlation between secondary oxidation products and yellowness what showed that accumulation of secondary oxidation products contribute to yellowness of fish flesh. Superchilling has a potential for prolonging the

shelf life of the mackerel products. Even though, the results of peroxide value and TBARS were significantly higher for superchilled fish, they were below the approved by FFA range (10 meq O_2/ kg oil) for human consumption. Optimization of the process and storage conditions of the product is needed in order to retain good quality of the product. Freezing seems to be a better option than prolonged chilled or superchilled storage, because the lipids are relatively stable and shelf life can be extended up to a year.

The analysis of sous-vide cooked mackerel fillets revealed that temperature and time of cooking had rather low influence on the formation of primary and secondary oxidation products. However, PV gradually increased with duration of chilled storage, which leads to the conclusion that storage duration has the largest effect on lipids stability of sous-vide cooked mackerel. Increase in TBARS and yellowness for sous-vide cooked mackerel and significant differences between analyzed mackerel samples show that increase in cooking temperature, time of exposure to heat and duration of chilled storage increase the content of secondary lipid oxidation products. Sous – vide cooking is a good option for preparing ready – to – eat products, because it can prolong the shelf life of the product. It can be also used in catering or restaurants for products that will be heated before serving, however optimal temperature and time of cooking are needed to maintain a high quality.

Acronyms

Abs Absorbance

CD Conjugated dienes

CHD Coronary Heart Disease

CVD Cardiovascular disease

DHA Docosahexaenoic acid

DPA Docosapentaenoic acid

EDTA Ethylenediaminetetraacetic Acid

EFAs – Essential Fatty acids

EPA Eicosapentaenoic Acid (20:5n-3)

FFA Free Fatty Acid

FRS Free Radical Scavenger(s)

Hb Heme

LA Linoleic Acid (18:2n-6)

LMW Low molecular weight

MDA Malondialdehyde

MUFA Monounsaturated Fatty Acid

PUFA(s) Polyunsaturated Fatty Acid(s)

ROS Reactive oxygen species

PV Peroxide Value

SD Standard deviation

SEM Standard error of mean

SFA Saturated fatty acid

TBA Thiobarbituric acid

TBARS Thiobarbituric acid reactive substances

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1. Introduction

1.1. Background

Fish, seafood and aquaculture are big and important sources of food and nutrition for people all over the world. 3% of world's food consumption comes from the ocean. The amount of proteins derived from the ocean is even higher than for foods (6 – 7%). The intake of marine food and proteins varies from country to country and it is expected to increase in the future. Fisheries and aquaculture constitute a big part of economy which provides jobs and income for millions. Seafood business provides: flow of foreign currency for developing countries, employment, food and nutrition supply. In 2014 developing countries earned US$80 billion from fish export, what is more than from other sectors of the economy combined (tobacco, rice, sugar) (FAO 2016). In 2014 world fish supply reached the highest level; around 20 kg fish per capita, and in 2013 in industrialized countries fish consumption per capita was 26,8 kg. (FAO 2016). The trade of seafood merchandise in many countries is still growing and more than 50% of fish exports come from developing countries. In 2014 global total capture fishery production reached 93,4 million tons (FAO 2016).

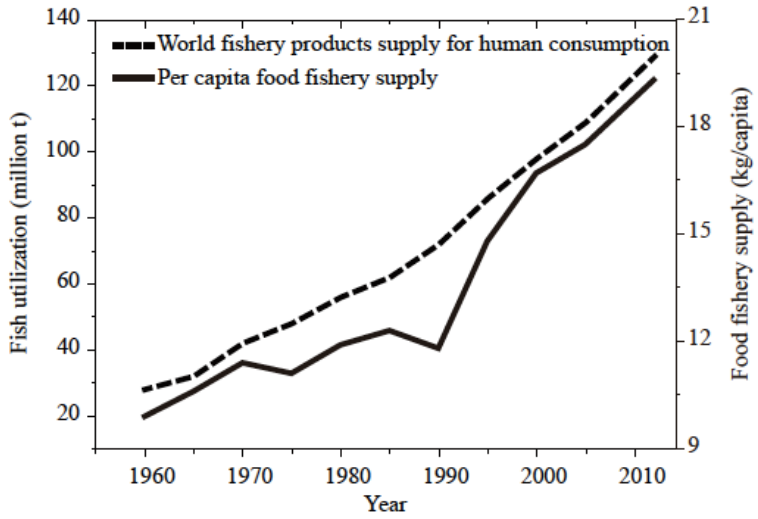

Figure 1.1: World fish utilization and supply (Wu, Yuan et al. 2014).

The main producers in the global market are, in order: China, Indonesia, United States of America and the Russian Federation (FAO 2016).

In 2014 more than 146 million tons, which is approximately 87% of global fish production, was intended for direct human consumption. Fish do not have to be consumed directly, as a fresh or chilled/frozen product. On the market there is a lot of processed products such as dried, smoked, cured or salted (Eymard, Baron et al. 2009). The remaining 21 million tons was used for non-food products such as fish oil, fishmeal or as raw material for feeding in aquaculture (FAO 2016). Thanks to improvement of living standards consumers more often demand and buy fresh aquatic products, which are characterized by high value, marketing and advanced supply chain (Wu, Yuan et al. 2014). They want convenient, easy to prepare, tasty products. Increased fish and fish products consumption will improve the diet by increasing the amount and diversity of consumed nutrients. Fish and fish products are a good source of high vale nutritional ingredients and easily digested animal proteins, which contain all essential amino acids. They are a good source of essential fats, such as long chain omega-3 fatty acids, with beneficial influence on human health. There are many health benefits from consumption of unsaturated fats including reduction of the risk of cardiovascular diseases, aids nervous system, fetal and infant development of the brain. Fish have low cholesterol and are relatively low in calories, depending on the fat content (Wu, Yuan et al. 2014). Fish also provide vitamins (A, B, D) and many minerals necessary for many body functions (calcium, iodine, zinc, iron and selenium), especially when eaten whole (FAO 2016). Increased fish intake can help to deal with unbalanced diets and in countering obesity (FAO 2016). Even though fish have a lot of beneficial effects for the human body, the consumption of it is still too low in many countries, especially in the Western world (Eymard, Baron et al. 2009).

Based on the reports, FAO estimated that the maximum volume of sustainable wild-caught fish has already been reached and 25% of the resources is already overexploited. Two solutions to this problem were proposed: greater use of other "new" species or increase in fish farming (Magnussen, Haugland et al. 2008). More than 100 million tons of fish and shellfish are caught every year and about 25% of this catch is turned into the waste due to microbial activity and chemical deterioration, what contribute to 25% of total loss of agricultural and fishery products annually (Kaale, Eikevik et al. 2011). Fish are the largest group of animals used for food production. Their kingdom is more than 30 000 already known species, but only about 700 are caught commercially and used to produce food on a large scale (Alasalvar 2010). Generally, around 50 - 60% of the catch is used, and the rest is wasted (Rustad 2006).

Despite of many positive aspects related to marine products there is one, big drawback. Fresh fish, shellfish and other aquatic products are highly perishable. They contain endogenous enzymes which promote proteolysis of proteins in the muscles and connective tissue. They can

also promote fat hydrolysis, which results in free fatty acids and promotes lipid oxidation (Wu, Yuan et al. 2014).

All of the aspects listed above shows the importance of preserving the nutritional value, and sensory quality of fish through storage and processing. There is a high demand for development of efficient preservation methods and technologies in order to extend shelf life retaining all the properties of fresh product: nutritional value, flavor, texture and quality. One of the biggest challenges is maintaining a stable and low temperature during distribution, which will preserve quality and prevent spoilage (Wąsowicz 2004). The temperature control and storage at the chilled temperature or freezing are efficient methods for maintaining nutrients, quality and prolonging shelf-life of the fish.

1.2. Nutritional value and health benefits

Fish and fish products are well known for their health benefits due to a high level of n-3 fatty acids, which are essential compounds involved in many physiological processes (Rui Pedrosa 2014). An increased consumption of fish and their products is therefore recommended.

Fatty fish species are abundant in long chain omega-3 polyunsaturated fatty acids (PUFA): EPA (eicosapentaenoic acid, C20:5 n-3) and DHA (docosahexaenoic acid, C22:6 n-3) (Eymard, Baron et al. 2009) and it is their consumption that brings the most beneficial effects.

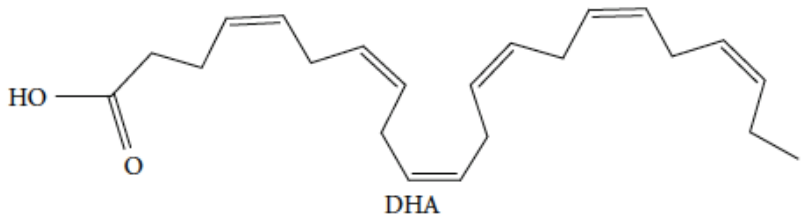

Figure 1.2: Chemical structure of docosahexaenoic acid (DHA) (D'Antona, Nabavi et al. 2014).

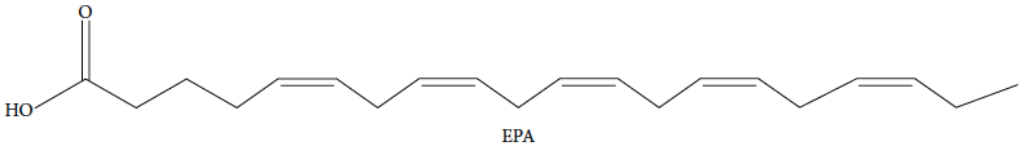

Figure 1.3: Chemical structure of eicosapentaenoic acid (EPA) (D'Antona, Nabavi et al. 2014).

Humans are able to elongate the chain of polyunsaturated fatty acids, but they are not very efficient in making long ω-3 chains from α-Linoleic acid. Thus, dietary supplementation by

EPA and DHA with for instance fish oil (available on the market as capsules or in free form as oil) or eating fatty fish is strongly recommended. Recommended intake of n – 3 PUFA per day is around500 mg (Rui Pedrosa 2014).

Fish contain long chain PUFAs (C18, C20 and C22) including linoleic acid (LA) [18:2 (n-6 omega)] and a -linolenic acid (a -LN) [18:3 (n-3 omega)] – two essential fatty acids (EFAs).

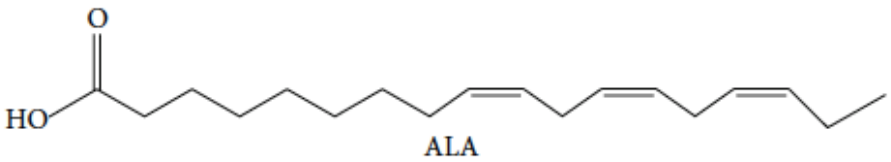

Figure 1.4: Chemical structure of α-linolenic acid (ALA) (D'Antona, Nabavi et al. 2014).

EFAs are involved in production of longer chain PUFAS – prostaglandins, which control many body functions, for instance blood vessels by dilation of peripheral blood vessels what results in lower arterial pressure (Gogus and Smith 2010).

Lipid content, specifically unsaturated fatty acids composition of the diet and lipid homeostasis have influence on many diseases: cardiovascular diseases, cancer, neurological disorders, obesity and diabetes, etc. (Rui Pedrosa 2014). Lipids take a part in hormonal synthesis and in several signal transduction pathways. The omega-6 and omega-3 PUFAs take part in lipid mediators (eicosanoids) formation. Eicosanoids regulate inflammation, for instance those derived from omega – 6 PUFAs, like arachidonic acid, exhibit immune – active and pro – inflammatory properties. n-3 PUFAs eicosanoids (EPA, DHA) have anti – inflammatory properties (Rui Pedrosa 2014) and they lower the risk of thrombogenesis and inflammation which can be caused by n-6 omega PUFAs (Gogus and Smith 2010). n-3 omega PUFAs can be used in the treatment of many diseases, for example eczema, psoriasis, inflammatory bowel disease (IBD), or rheumatoid arthritis (Gogus and Smith 2010).

It is highly recommended to supplement the diet with n-3 omega PUFAs for protection against cardiovascular diseases (CVDs) (Romotowska, Gudjónsdóttir et al. 2016). Increased intake of n-3 omega PUFAs derived from marine products, such as flesh of fatty fish, livers of lean fish can decrease triglycerides, platelet and fibrinogen aggregation and can reduce the risk of CHD (Turner, McLean et al. 2006, Gogus and Smith 2010). n-3 omega PUFAs lower the total cholesterol level and can affect psychological health (Romotowska, Gudjónsdóttir et al. 2016, Gogus and Smith 2010). Low fish consumption can be correlated with mental conditions, for example with depression (Turner, McLean et al. 2006, Romotowska, Gudjónsdóttir et al.

2016) and that phospholipids can have a beneficial effect on the treatment of schizophrenia (Gogus and Smith 2010).

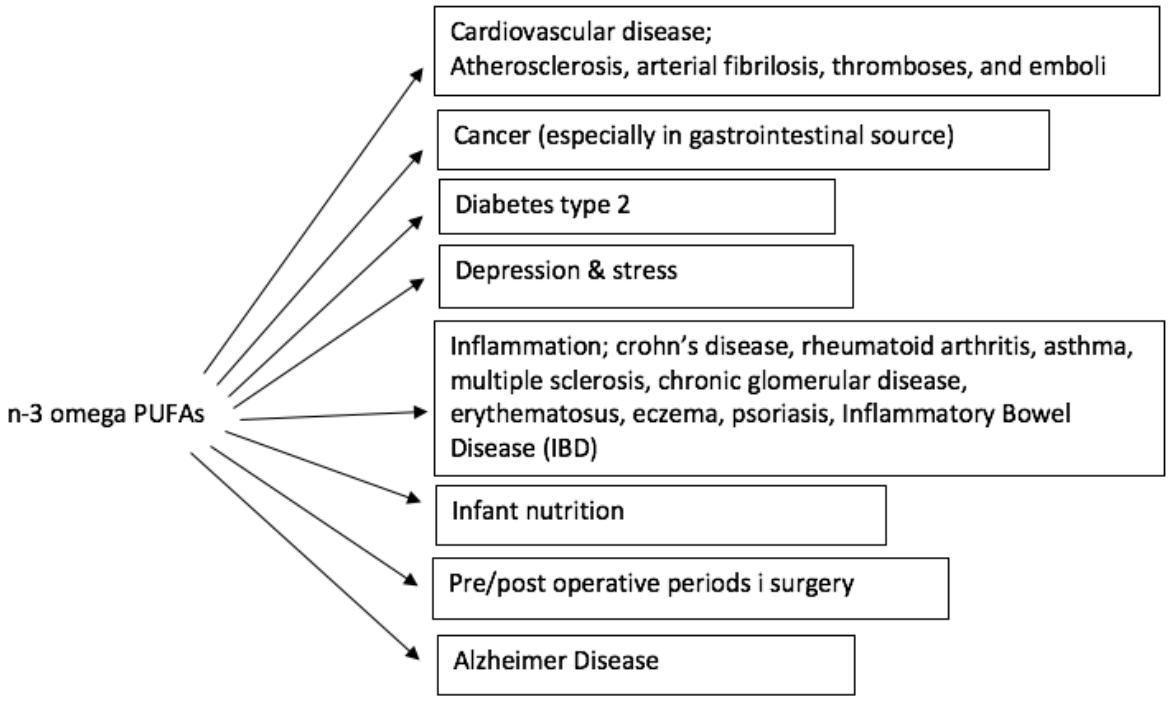

Figure 1.5: Current application of n-3 omega polyunsaturated fatty acids on human health (Gogus and Smith 2010).

1.3. Objectives of this study

This master thesis is a part of the project ProHealthPelagic which is a collaboration between four countries: Norway (SINTEF Ocean and NTNU), Ireland (Agriculture and food authority – Teagasc), Italy (University of Perugia) and Poland (National Marine Fisheries Research Institute – NMRFI).

The main goals of this project are:

- to preserve the health beneficial effects in pelagic fish products through the whole processing chain,
- understand the effects of processing methods on preserving safety and quality with focus on healthy nutrients,
- develop strategies of communication to spread the results to industry, society and academia.

The main objective of this master thesis was to study and understand how different types of storage influence quality and stability of lipids of Atlantic mackerel (*Scomber* scombrus) and

to find processes that retain the healthy omega-3 fatty acids. The aim of was to evaluate the effect of cold preservation (chilling, superchilling and freezing) on the stability and quality of raw material. Amount of lipid was determined using standard solvent extraction methods and oxidation status was evaluated by measurements of peroxides and thiobarbituric acid reactive substances. Additionally, influence of sous-vide (light heat treatment) and further chilled storage on mackerel lipids was studied by evaluation of primary and secondary products of lipid oxidation. Color parameters (yellowness, redness and lightness) were assessed in order to evaluate correlation between formation of oxidation products and color development.

1.4. Atlantic mackerel (*Scomber Scombrus*)

Atlantic Mackerel (*Scomber Scombrus*) belongs to the family Scrombiadae (Haque and Chun 2016). It is a pelagic fish that inhabit cold and temperate shelf areas and it is common from Northwest Africa to the Barents Sea and to the west of Iceland in the Norwegian Sea (nifes.hi.no). Mackerel migrates during the lifespan in large schools in search of food and to restore energy reserves (Romotowska, Gudjónsdóttir et al. 2016). Mackerels are elongated, fast-swimming fish with steel-blue "tiger" stripes along the back and a long, pointed snout. The mature mackerel is typically 30 – 40 cm long.

Figure 1.6: Mackerel. Picture adapted from sjomatdata.nifes.no.

Mackerel as fatty fish has a high content of omega-3 PUFAs. It is also a good source of proteins, essential amino acids and other biologically active compounds (Haque and Chun 2016). Among the nutrients common for this species are: 0,0046 milligram/100 grams fish of Vitamin D, 0,020 milligram/100 g fish of Iodine and 3550 milligram/100 gram of EPA and

DHA. Atlantic mackerel is considered as safe to eat because it does not contain large amounts of heavy metals or organic pollutants (POPs) (nifes.hi.no).

Mackerel have a large proportion of dark muscles. Sudden, quick movements are sustained by the light muscle, while short bursts of energy come from dark muscles. Dark muscle has more lipids than light and are characterized by seasonal variations in fat content. Muscle lipid content usually increases during periods abundant in food what result in remarkable variations, both, seasonal and individual in the fatty acid composition and quality (Romotowska, Gudjónsdóttir et al. 2016).

Mackerel is sold and exported as frozen (whole or headed/gutted) fillets, smoked, canned and spread in tomato sauce (Romotowska, Gudjónsdóttir et al. 2016). Because of the high content of PUFAs mackerel is considered as highly perishable food and demand appropriate treatment to prevent deterioration (Standal, Mozuraityte et al. 2018). The shelf life of the fish during storage (chilled or frozen) depends on many factors, where compositional differences (levels of PUFAs/antioxidants/pro-oxidants) and the temperature from the catch to the consumer are very important (Standal, Mozuraityte et al. 2018).

1.5. Lipid/fatty acid composition of mackerel

Lipids are present in all cells, being a vital nutrient with many functions such as essential macromolecules of organelle and cellular membranes, body energy reserves, important fuel molecules etc. (Rui Pedrosa 2014). They are used in many processes, for instance in: signal transduction pathways, transport, hormonal synthesis or in the absorption of fat-soluble vitamins (Rui Pedrosa 2014). Lipids are also associated with flavors and aromatic substances, they are crucial for food, food processing and cooking (Rui Pedrosa 2014). Lipids are a large and diverse group of compounds, which are insoluble in water and highly soluble in organic solvents like: chloroform, benzene, hexane, etc. Fatty acids include carboxylic acids with hydrocarbon chains 4 - 36 carbons long (C4 - C36). They can be fully saturated or unsaturated with one (monounsaturated fatty acid—MUFA) or several (polyunsaturated fatty acid—PUFA) double bonds. The unsaturated fatty acids can be divided into three groups: ω -3 (omega 3/n-3), ω -6 (omega 6/n-6) and ω -9 (omega 9/n-9) (Rui Pedrosa 2014).

Table 1.1: The list of PUFAs *(Gogus and Smith 2010).*

Name	Lipid name	Chemical name
α-Linolenic acid (α-LN)	18:3 (n-3)	all-*cis*-9,12,15-ortadacatrienoic acid
Stearidonic acid (STD)	18:4 (n-3)	all-*cis*-6,9,12,15-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (n-3)	all-*cis*-11,14,17-eicosatrienoic acid
Eicosatetraenoic acid (ETA)	20:4 (n-3)	all-*cis*-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA)	20:5 (n-3)	all-*cis*-5,8,11,14,17-eicosapentaenoic acid
Docosapentaenoic acid (DPA)	22:5 (n-3)	all-*cis*-7,10,13,16,19-docosapentaenoic acid
Docosahexaenoic acid (DHA)	22:6 (n-3)	all-*cis*-4,7,10,13,16,19-docosahexaenoic acid
Tetracosapentaenoic acid	24:5 (n-3)	all-*cis*-9,12,15,18,21-docosahexaenoic acid
Tetracosahexaenoic acid	24:6 (n-3)	all-*cis*-6,9,12,15,18,21-tetracosenoic acid

Fish species are grouped into four categories according to fat content: lean fish (<2% of total fat), low-fat (2–4% of total fat), medium-fat (4–8% of total fat) and high-fat or oily fish (>8%) (Rui Pedrosa 2014). In mackerel (oily fish) the fat content can vary between 1,8 to 23%, with large differences between dark and light muscles, with the highest values in September/October (Rui Pedrosa 2014, Standal, Mozuraityte et al. 2018). The lipid content in fish - amounts of the omega-3 PUFA is dependent on many conditions such as diet, food availability, species, season, water temperature, age, size, sex, maturation status or geographical location (Rui Pedrosa 2014, Turner, McLean et al. 2006).

As mentioned earlier, pelagic fish (including mackerel) in general is well known for its high content of essential omega-3 polyunsaturated fatty acids, particularly DHA and EPA which are known from benefits for human health (Maestre, Pazos et al. 2011). The content of EPA and DHA in recent studies was approximately 7% and 10 % (w/w), respectively, of the total fatty acids what corresponds to ca 1.26 g EPA and 1.80 g DHA pr 100 gram fillet (Standal, Mozuraityte et al. 2018). The most abundant fatty acid was DHA which constituted around ca 15% of total fatty acids. The fatty acids composition also varies according to season and geographical origin (Standal, Mozuraityte et al. 2018).

1.6. Lipid oxidation and lipid hydrolysis

As mentioned previously, foods and marine lipids are a good source of very–long– chain n-3 PUFAs but, at the same time, they are easily oxidized during storage and processing (Turner, McLean et al. 2006). Lipid oxidation is a huge problem for the food industry because of undesirable and toxic compounds created during the process, color deterioration, quality loss due to changes in taste, texture, appearance as well as limiting the shelf life of foods (Rui

Pedrosa 2014, Romotowska, Gudjónsdóttir et al. 2016, Maqsood, Benjakul et al. 2012). Pelagic fish are especially susceptible to lipid oxidation because of a large proportion of dark muscles and thus high level of PUFAs and heme. Oxidation of fish lipids starts when fish is still alive due to capture stress (Turner, McLean et al. 2006). Products formed during lipid oxidation can react with nitrogenous materials in biological systems such as proteins, phospholipids, amino acids and DNA, what results in formation of brown pigments and fluorescent compounds, which can have negative effects on human health (Turner, McLean et al. 2006).

Oxidation in fish can be influenced by external factors such as storage, temperature, time, precooking, processing, or packaging, as well as by internal factors, like composition of muscle (Maestre, Pazos et al. 2011). Lipid oxidation of fish and aquatic products, and further interactions between lipid-oxidized products and proteins can lead to cross-linking of proteins (including amino acids), phospholipids and DNA, that results in toughening, discoloration and negative health effects (Wu, Yuan et al. 2014). Lipid peroxidation can cause various diseases such as atherosclerosis, cataract, rheumatoid arthritis, neurodegenerative disorders (Niki, Yoshida et al. 2005) or thrombosis (Turner, McLean et al. 2006). Due to this, it is extremely important to understand the process of lipid oxidation and minimize/control lipid oxidation during storage and food processing (Romotowska, Gudjónsdóttir et al. 2016).

Oxidation of highly unsaturated fatty acids can be caused by photo-oxidation, autoxidation or enzymatic mediated oxidation, and can be triggered by free radicals (hydroxyl radical; peroxyl radicals) or non-free radical oxidants, for example singlet oxygen (Rui Pedrosa 2014). Each type of oxidation yields specific products such as hydroperoxides, aldehydes or polymeric materials, which can have cytotoxic or genotoxic effects (Niki, Yoshida et al. 2005). The primary product formed during lipid oxidation is an odorless hydroperoxide (Guillén-Sans and Guzmán-Chozas 1998). Autoxidation can take place by the classic, free radical mechanisms (in the dark) or via the photo – oxidation mechanism, which requires light for initiation, or via the lipoxygenase mechanism (Guillén-Sans and Guzmán-Chozas 1998). Hydroperoxide is highly unstable and reacts further creating secondary products, such as aldehydes, ketones and hydrocarbons, that leads to the formation of off-color, off-flavors and have influence on vitamins (Rui Pedrosa 2014). When the decomposition into secondary products exceeds the formation rate, the level of hydroperoxides decreases. During lipid oxidation the primary oxidation products are formed in the early oxidation phase, while the secondary oxidation products accumulate in the more advanced phase of the process.

Three stages can be distinguished during oxidation of lipids: initiation, propagation and termination (Rui Pedrosa 2014). Initiation stage, shown in equation 1.1 and 1.2 can be enhanced by heat, light, lipolytic enzymes or metal ions (Rui Pedrosa 2014).

$$RH + O_2 \rightarrow R\cdot + HOO\cdot \quad (1.1)$$

$$RH \rightarrow R\cdot + H\cdot \quad (1.2)$$

Free radical (R·) is formed from free fatty acid molecule or triglyceride. Further, it reacts with oxygen and form peroxide radical which is needed to produce hydroperoxides - propagation step (equation 1.3 and 1.4) and new free radicals which reinitiate the process (Guillén-Sans and Guzmán- Chozas 1998).

$$R\cdot + {}^3O_2 \rightarrow ROO\cdot \quad (1.3)$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot \quad (1.4)$$

The process stops when two radicals form inactive substance (termination stage, equations 1.5 and 1.6) (Guillén-Sans and Guzmán-Chozas 1998).

$$R\cdot + R\cdot \rightarrow R - R \quad (1.5)$$

$$ROO\cdot + R\cdot \rightarrow ROOR \quad (1.6)$$

The reactive oxygen species (ROS) include oxygen radicals and non-radical derivatives of oxygen, for example: superoxide (O_2^{-}), hydroxyl (OH·), peroxyl (RO_2), alkoxyl (RO·), peroxy (ROO·), hydroperoxy (HOO·), hydrogen peroxide (H_2O_2), hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen (Choe and Min 2006). Living organisms have anti-oxidative mechanisms, which defend the organisms against ROS, but this is not working after death resulting in accumulation of ROS in the muscle tissue (Tokur and Korkmaz 2007). Accumulation of ROS in muscles leads to lipid oxidation and is a major problem in fatty fish storage and processing, particularly in dark muscles of pelagic fish species because of presence of pro-oxidants like heme proteins (Hb), myoglobin, transition metal complexes with low molecular weight (LMW) etc. in dark muscles (Tokur and Korkmaz 2007). Heme compounds are well distributed in biological systems with especially high concertation in red blood cells. Hemoglobin and myoglobin carry and distribute oxygen to different tissues in the body. Hemoglobin is the main pigment in red blood cells, while myoglobin is the main pigment in muscle cell (Baron and Andersen 2002). Hemoglobin and myoglobin are believed to be one of the most important promoters of lipid oxidation in fish muscle (Maqsood, Benjakul et al. 2012).

ROS can react with biological molecules such as lipids, proteins, sugars and vitamins, and can catalyze oxidative reactions which can accelerate the quality of fish and fish products (Baron, Kjaersgård et al. 2007). ROS can be considered as dangerous for human health, they can produce low-molecular-weight volatile aldehydes, alcohols, hydrocarbons, carcinogens, change the functionalities of lipids, proteins and carbohydrates and destroy essential nutrients (Choe and Min 2006).

1.7. Oxidative stability and methods of analysis:

There are many techniques and analytical protocols used for evaluation of the effects of lipid oxidation in food. In order to assess the degree of oxidation of lipids primary and secondary oxidation products can be analyzed by relatively easy and common methods, like PV and TBARS, respectively. Besides the common and simple methods, more advanced techniques such as fluorescence or chemiluminescence probes, ESR spin trapping technique, ion-spray mass spectrometry (CIS-MS), electrospray ionization (ESI) or matrix-assisted laser desorption an ionization time-of-light (MALDI-TOF) mass spectrophotometry can be also used, in order to evaluate lipid oxidation (Niki, Yoshida et al. 2005).

1.7.1. Primary oxidation products

Lipid hydroperoxides are formed as the primary oxidation products. They are unstable and undergo secondary reactions (Niki, Yoshida et al. 2005). There are several methods to determine peroxide value (PV), for example photometric method used in evaluation of PV for chilled, superchilled and frozen fish or titration method used for mackerel after sous – vide cooking.

1.7.1.1. Peroxide value (PV)

For determination of primary oxidation products peroxide value (PV) can be determined by photometric method. PV is a good indicator of the level of lipid, fat and oil oxidation, and its main advantage is, that it directly measures the lipid peroxides (Shantha 1994). The method is based on the capability of lipid peroxides to oxidize ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) at

low pH. After addition of ammonium thiocyanate, peroxides present in oil oxidize, the ferric ions react and give red colored complex. Formed ferric thiocyanate complex can be determined by photometric determination at 500 nm (Shantha 1994). PV value is expressed in milliequivalents peroxide per kg oil (meq O_2/kg). The method is sensitive and reproducible, can detect maximum PV of 40 meq O_2/kg or minimum of 0,1 meq O_2/kg (Shantha 1994).

1.7.1.2. Iodometric titration

Another method used for determination of primary oxidation products is iodometric titration method which is a common method described in AOCS official methods (Cd 8b-90). Iodometric titration determines all substances, generally assumed to be peroxides or similar products of lipid oxidation, which oxidize potassium iodide under the test conditions and results are given in milliequivalents of peroxide per 1000 grams of sample. This method can be used for all fats and oils (AOCS).

1.7.1.3. Conjugated dienes (CD)

In order to detect primary lipid oxidation products UV measurement for determination of conjugated dienes (CD) can be used. However, the PV method is preferred in the industry, because it measures the value of hydroperoxides in the early phase of oxidation (Fennema O.R. 2007). The method itself is less specific and less sensitive than PV, however it is much faster, requires only a small amount of sample and no other reagents (Shahidi and Zhong 2005).

1.7.2. Secondary oxidation products

As mentioned earlier, the primary products of oxidation break down into the secondary oxidation products, namely into ketones, aldehydes, epoxides, hydroxyl compounds, oligomers and polymers. The ketones and aldehydes are responsible for unpleasant odors. The most abundantly formed aldehyde is malondialdehyde (MDA) (Barriuso 2012). Over time secondary oxidation products break down into short-chain tertiary products (Turner, McLean et al. 2006).

1.7.2.1. Thiobarbituric reactive substances (TBARS)

Thiobarbituric reactive substances (TBARS) method can be used in analysis of any type of food, with one exception, except in fried foods. 2-Thiobarbituric acid (TBA) is a widely used compound, which easily reacts with carbonyl substances, such as aldehydes and ketones. Acids, esters, amides, sugars, and pyrimidine compounds also react with TBA (Guillén-Sans and Guzmán-Chozas 1998). Reaction of C=O group can occur thanks to the ability of the methylene group in the C-5 position on the molecule. Formed products have a polar double linkage (Guillén-Sans and Guzmán-Chozas 1998). A pink complex is formed as a result of interaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), and it can be determined spectrophotometrically at 530-535 nm (Shahidi and Zhong, 2005).

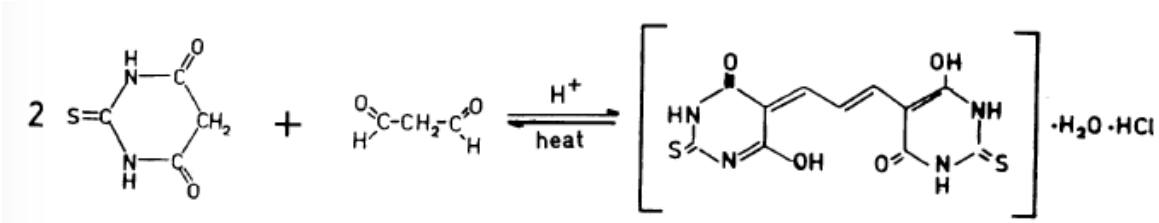

Figure 1.8: Reaction between TBA and MDA in TBA assay (Guillén-Sans and Guzmán-Chozas 1998).

TBA value represents the level of aldehydes in the lipids which were autoxidized and is a parameter for the secondary oxidation step (Guillén-Sans and Guzmán-Chozas 1998). It is expressed as μmoles TBARS/g sample. The method is reproducible but lacks sensitivity. However, even with the limitations, it is still one of the most common used techniques and is a good method for determination lipid oxidation in foods (Shahidi and Zhong, 2005). The reaction conditions are very important for the method. Temperature, pH, presence of antioxidants and metals, etc. have a large influence on color development. Nevertheless, the main limitations result from possibility of reactions between other components and TBA, that can contribute to the color development (De las Heras A. 2003).

Besides the TBA assay there are many other methods to determine secondary oxidation products, for example UV-Vis spectrophotometry for detection of presence of aldehydes-p-anidisine value, which is used in the industry. Volatiles, which include aldehydes, ketones, alcohols, short carboxylic acids and hydrocarbons are responsible for deterioration of flavor. They can be quantified by gas chromatography and identified by mass spectrophotometry (Barriuso 2012).

1.8. Antioxidants and pro-oxidants

Various factors can influence the rate of lipid oxidation, including:

- processing and storage conditions, where the factors are: temperature, light, oxygen, enzymes and metals;
- fat content and amount of unsaturated fatty acids (dark muscles) and distribution of PUFAs in triacylglycerol molecule;
- presence of antioxidants, which are inhibitors of oxidation; and presence of prooxidants, which are catalysts (Wąsowicz 2004).

Lipid oxidation can be inhibited by different types of antioxidants. The reduction of lipid hydroperoxides prevents further oxidation, decomposition of fats and formation of free radicals. The cells have a programmed system based on the activity of different antioxidant enzymes, responsible for the reduction of lipid hydroperoxides (Niki, Yoshida et al. 2005). Antioxidants can prevent the formation of active oxidants and enzymes. They can work as radical scavengers (for example vitamin C) in order to prevent auto-oxidation or as oxygen quenchers (for example flavonoids) to prevent photo-oxidation. Antioxidants can also create adaptive responses, for example chelation of pro-oxidative metals, like citric acid (Niki, Yoshida et al. 2005, Turner, McLean et al. 2006).

In order to prevent PUFAs from undergoing rapid lipid oxidation, hydrophobic antioxidants (Vitamin E or Co-enzyme Q10) can be used. They can be used as dietary supplement or can be added during the production process of foods (Rui Pedrosa 2014). Both, vitamin E and Co-Q10 react with peroxyl radicals and form non-radical products what prevents from further reactions. Co-Q10 is a part of all cells that have mitochondria, what makes fish and meat the main source of this antioxidant (Rui Pedrosa 2014). Antioxidants are commonly added to fish oils in order to prolong the shelf life of the product and preserve organoleptic properties (Turner, McLean et al. 2006).

Fish have their own endogenous antioxidant system. The loss of endogenous a-tocopherol can refer to the beginning of propagation stage of lipid oxidation. When the fish is dead, other endogenous antioxidants, such as ascorbic acid are lost (Maestre, Pazos et al. 2011) and the oxidation rate increase.

1.9. Lipid hydrolysis and free fatty acids

Lipid deterioration can also occur via lipid hydrolysis. In order to evaluate lipid hydrolysis, amount of free fatty acids (FFA) have to be determined. FFA are formed as a result of enzymatic action of lipases and phospholipases (endogenous enzymes) (Bernardez, Pastoriza et al. 2005). Hydrolysis of triacylglycerol (TGA) leads to formation of monoacylglycerols (MAGs) and diacylglycerols (DAGs) and free fatty acids (Huss 1995). FFA development during processing and storage have negative influence on the quality of fish and fish products. It has been shown that during chilled storage the content of FFA increase with time (Kolakowska 2011). FFA accumulates mostly in the dark muscle tissue. FFA formation does not contribute to the nutritional loss, however its accumulation is related to the negative effects on ATPase, protein solubility and texture. It also enhances the formation of off-odors and off-flavors (Fennema 2007). Lipid hydrolysis can either accelerate or inhibit the lipid oxidation, depending on the origin of free fatty acids (Shewfelt 1981).

1.10. Cold storage preservation

Quality can be lost in every step of food processing, from harvest through handling, processing, packaging to storage. Food industry has to deal with challenges related with preservation and distribution of fresh foods in order to maintain high quality and nutritional value (Eymard, Baron et al. 2009). Temperature control is highly important for preservation of quality; rate of the spoilage and shelf life of fresh vegetables, fruits, fish, pork, poultry, etc. (Magnussen, Haugland et al. 2008).

Nowadays, the most important methods of food preservation and distribution is chilling and freezing (Magnussen, Haugland et al. 2008). Unfortunately, chemical and enzymatic oxidation of lipids, which leads to rancidity also takes place at low temperatures. Moreover, maintaining stable, low temperature during distribution and storage is more challenging in fresh, than for frozen foods. The shelf life of chilled or frozen mackerel fillets depends on several factors, for instance temperature history after catch and compositional differences (PUFAs/antioxidants/pro-oxidants) (Standal, Mozuraityte et al. 2018).

Even though, there has been some research done on influence of cold storage on lipid stability, the lipid oxidation process should be studied more in order to fully understand the

behavior and mechanism of lipid oxidation of fish muscle during chilled, superchilled and frozen storage.

1.10.1. Chilling

As temperature is one of the most important factors influencing quality and safety of fresh foods, it is extremely important to keep the temperature low during storage in order to inhibit food deterioration. Marine products, like fish and shellfish, kept in chilled storage are not completely protected from microorganisms and enzymes activities, which are still active. Both of these factors lead to deterioration. Refrigeration temperature can slow down the growth of microorganisms and reduce chemical and enzymatic reactions (Turner, McLean et al. 2006). In Norway the main part of the fish harvests is preserved by refrigeration, while the rest is preserved by drying, canning and salting methods. Modern storage plants are equipped in energy efficient refrigeration systems, which are friendly for environment, as well as in chilling and freezing equipment and cold storage facilities (Magnussen, Haugland et al. 2008).

1.10.2. Superchilling

The superchilling process was described for the first time in 1920 by Le Danois (Kaale, Eikevik et al. 2011). Superchilling was used in Portugal from 1963, fish were stored at -2 to -5ºC through circulating seawater on deep-sea trawlers. In China refrigerated seawater (RSW) was used for superchilling resulting in the extending shelf life to more than 20 days (Wu, Yuan et al. 2014).

Superchilling/deep-chilling/partial freezing/partial ice formation is a process, where about 10 – 30% of the water content of the product is frozen. During the process the temperature is lowered 1 – 2 °C below the initial freezing point of the product, which is in the borderline between freezing and chilling. As a result, the surface is freezing in the outer few millimeters, and the product obtains an even, cold temperature inside (Magnussen, Haugland et al. 2008, Wu, Yuan et al. 2014, Duun 2008). The process has two stages: chilling and storage. During the first stage heat is removed rapidly. The process of superchilling can be carried out in different types of freezers: cryogenic freezers, impingement freezers or mechanical freezers

(Kaale, Eikevik et al. 2011). Cryogenic freezers apply either nitrogen liquid (-196°C) or carbon dioxide (-78°C) directly to the product to reach certain temperature (Kaale et al. 2011). Impingement freezers have big freezing chamber divided into zones. The impingement freezers have a good impact on the product quality and can be considered as the best way of preserving foods and extending shelf life (Kaale, Eikevik et al. 2011). Mechanical freezers use a circulating refrigerant to reduce the temperature of the product and they are commonly used to freeze foods. After partial freezing the product is immediately placed at chilled, storage temperature (Duun 2008). The internal ice left after equilibrium serves as a cold reservoir, absorbing the heat from environment keeping the product temperature stable during transportation or storage for a period of time (Magnussen, Haugland et al. 2008, Kaale, Eikevik et al. 2011). Storage temperature should remain as constant as possible because even small temperature variations can cause large variations in ice content and changes in the quality of superchilled product (Duun and Rustad 2008). Compared to traditional chilled storage, superchilling can extend the shelf life of the food 1,4 – 5 times (Duun 2008, Wu, Yuan et al. 2014), for example shelf life of pork roasts can be extended from 2 to 16 weeks (Duun 2008), shelf life of roast leg of pork can be more than doubled (Magnussen, Haugland et al. 2008) and shelf life of fish can be extended about 7 days (Duun and Rustad 2007, Duun 2008). Modified atmosphere packaging, coating preservation, vacuum packaging and cryoprotectants can be used together with superchilling (Wu, Yuan et al. 2014).

Superchilling technology keeps the food fresh, retains high quality of the products and terminates/inhibit growth oh harmful microorganisms. It is one of the most efficient methods to maintain high quality and can extend shelf life of foods compared to conventional chilling. In commercial and industrial processing superchilling can reduce energy use and costs, as well as negative impact on environment (Wu, Yuan et al. 2014, Kaale, Eikevik et al. 2011, Duun 2008). Despite the fact that this process has many advantages, it also has some drawbacks. Chemical and physical changes can progress during superchilled storage, and sometimes even accelerate. During superchilling increased drip loss can occur as a result of surface freezing of the product (Magnussen, Haugland et al. 2008). Hitherto the main challenges of superchilling technology are: selecting and maintenance optimal conditions (temperature, velocity).

Nowadays the process is successfully used for superchilling of: tilapia (*Oreochromis sp.*), goldfish (*Carassius auratus*), sea bass (*Lateolabrax japonicas*), sardina (*Sardina pilchardus*), grass carp (*Ctenopharyngodon idella*), penaeid shrimp (*Penaeus vannamei*), Atlantic salmon (*Salmo salar*), large yellow croaker (*Pseudosciaena crocea*), and turbot (*Scophthalmus maximus*) surimi products, etc. (Wu, Yuan et al. 2014).

1.10.3. Freezing

Freezing is the most common technique used for food preservation for muscle foods, some vegetables and fruits in order to prolong the shelf life of the foods (Romotowska, Gudjónsdóttir et al. 2016, Standal, Mozuraityte et al. 2018). 55% of total processed fish intended for human consumption and 26% of total fish production in 2014 was frozen (FAO 2016). Shelf life of frozen fish is much longer than that of fresh fish. Chilled mackerel fillets have shelf life around 9 – 10 days, highly dependent on temperature, while frozen can have a shelf life up to a year (Standal, Mozuraityte et al. 2018). Frozen products reduce the amount of food which becomes unacceptable for sale due to deterioration (Duun and Rustad 2008).

In Europe the temperature recommended for frozen storage is between -25°C to -30°C. This temperature range also applies in case of Atlantic mackerel fillets storage in Norway. It was shown that, storage of whole mackerel at lower temperature can increase protection against lipid oxidation and hydrolysis (Standal, Mozuraityte et al. 2018). Long term frozen storage of fish and fish products can have a negative effect on the quality (toughness and loss of functional properties of proteins), can lead to fatty acid oxidation and development of rancid odors (Baron, Kjaersgård et al. 2007). Ice formed during freezing and recrystallization can destroy the tissue, cause cell dehydration, drip loss and tissue shrinkage (Magnussen, Haugland et al. 2008, Kaale, Eikevik et al. 2011).

1.11. Sous vide

Sous Vide (SV) technology can be described as 'food cooked under controlled conditions of temperature and time inside heat-stable vacuum pouches' (Diaz, Garrido et al. 2011). Sous vide process is based on pasteurization/cooking at 65 - 85°C, over a certain period of time (up to 16 h), and rapid cooling right after cooking. Product obtained this way is ready to eat and while kept under refrigeration (0-4°C), preserved for a long time (4 – 9 weeks, depending on raw material and conditions during and after process). Sous vide products are widely used in catering (Diaz, Garrido et al. 2011), restaurants as products heated before serving and can provide consumer with ready-to-heat products. The sous – vide process is different from traditional cooking in two aspects: the temperature in sous – vide is precisely controlled and food is vacuum – sealed in heat – stable plastic pouches (Baldwin 2012). Vacuum – sealed pouches have several advantages: efficiently transfer heat to the product and eliminate the risk

of contamination during storage what prolong the shelf life. They inhibit development of off-flavors, reduce aerobic bacterial growth, prevent loss of flavor volatiles and moisture (Baldwin 2012). The sous – vide technique can be performed in two forms: cook – hold/cook – serve (product is served right after cooking) or cook – chill/cook – freeze (chilled/frozen product is stored for some time before serving) (Baldwin 2012).

The main advantages of sous vide over conventional cooking are: SV reduces heat damage to lipids and proteins, can enhance texture by increasing juiciness and tenderness, loss of moisture is lower and heat-sensitive nutrients remains practically untouched. Cooked fish is susceptible to slow physicochemical (hydrolytic and oxidative processes) and microbiological (anaerobic microflora) processes during storage, which lead to sensory spoilage. In SV cooked fish lactic acid bacteria can cause spoilage, produce rancid odors and off-taste. Presence of oxygen in the package can promote the development of yeasts and molds. Proteases and lipases, which are not degraded during the cooking can also cause spoilage of foods during chilled storage (Diaz, Garrido et al. 2011).

2. Materials and methods:

2.1. Overview

Characterization of five different parameters (total lipid content, PV, TBARS in chloroform phase, TBARS in methanol/water phase, color) of chilled, superchilled and frozen skin-on fillets of Atlantic Mackerel (*Scomber scombrus*) was performed in order to examine oxidative stability. The fillets were stored at different temperatures to study the effect of various types of storage on the quality of mackerel lipids. Additionally, the effects of various sous-vide time temperature regimes and their interactions on lipids stability in mackerel during chilled storage were determined.

2.2. Raw material, processing and sampling

The fish were caught January 21st, 2017 and landed at the processing plant of Pelagia A.S. in Selje (Norway) two days later, where it was filleted mechanically. The average weight of the resulting skin-on fillets was 89 ± 9,6 g and length was 17 ± 1,4 cm. Then, fillets were packed into two packages, 15 kg each and placed on ice. The bags were transported to Trondheim by boat on January 24th and landed there the next day - 25th, which was set as day 0 for sampling. Fillets were vacuum packed, four fillets per bag. Fish for chilled storage was kept at NTNU, at +4°C until sample collections. Another part of the fish was transported to SINTEF, where it was frozen and kept at -27°C. Fish intended for superchilling was frozen at -37°C for 1,5 min and then kept at -1,7°C, until sample collections. Samples and codes were prepared according to the table 2.1:

Table 2.1: Sampling codes with dates of collections.

Date	Day	Chilled	Superchilled	Frozen
25.01.2017	0	K03, K14, K16		
27.01.2017	2	K07, K10, K11	S01, S04, S05	F03, F11, F13
30.01.2017	5	K06, K09, K15	S07, S08, S15	
1.02.2017	7	K17, K18, K19		
3.02.2017	9		S06, S10, S14	
8.02.2017	14		S02, S09, S13	
27.09.2017	8 months			F02, F09, F10
19.01.2018	1 year			F01, F06, F12, F16

Sample collections were performed at different days. Chilled samples were prepared at day 0, 2, 5 and 7; superchilled at day 2, 5, 9 and 14; and frozen fish was taken after 2 days, 8 months and 1 year of storage. In order to perform primary and secondary oxidation products analysis fillets were minced with skin.

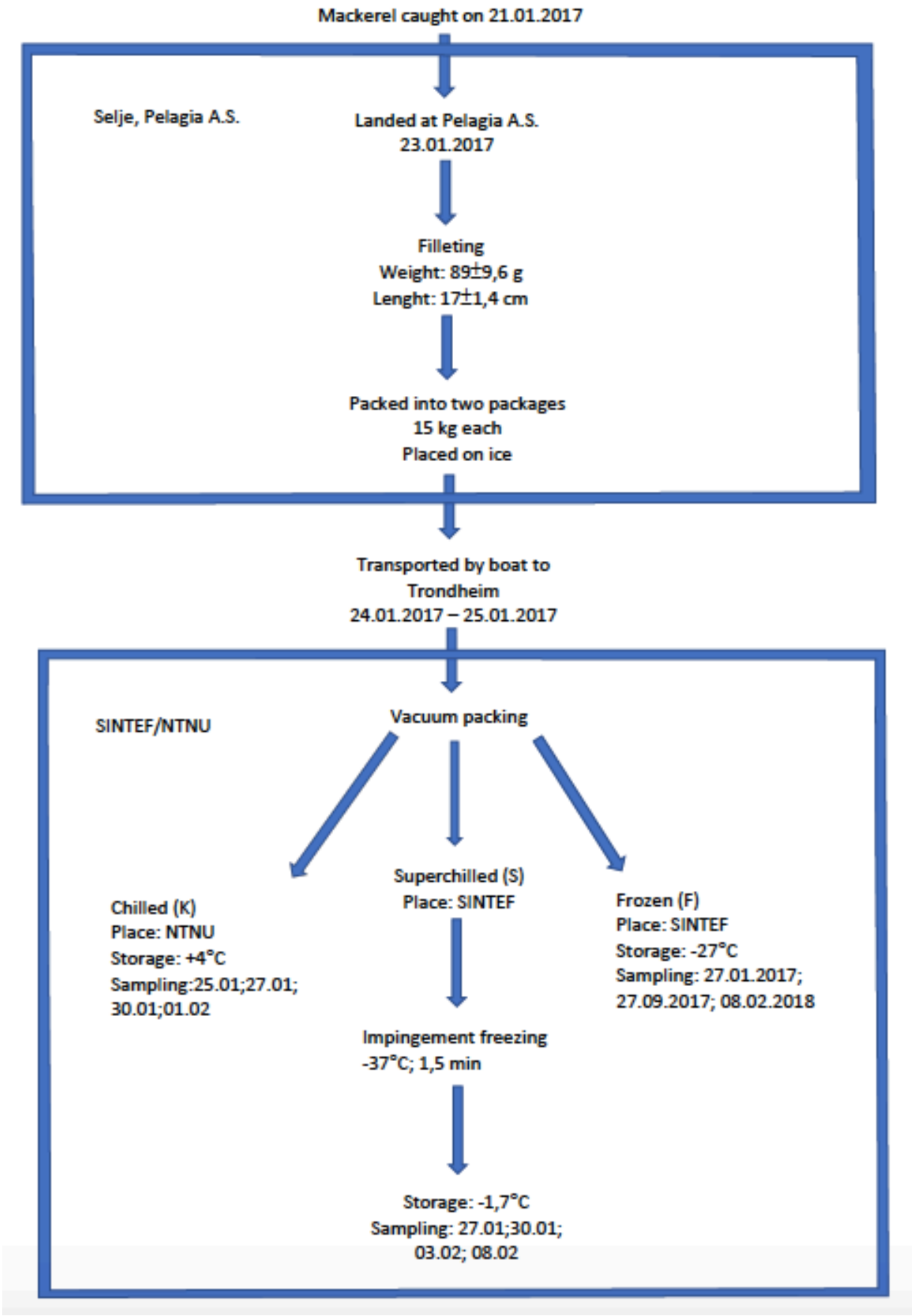

Figure 2.1: Scheme of the treatment of mackerel from catch to sampling, illustrated by Emilia Budzko.

In addition, the effect of various sous-vide time-temperature regimes and their interactions on quality parameters of Atlantic mackerel (*Scomber scombrus*) during chilled storage was evaluated. After the frozen fish were delivered from Pelagja A.S., they were thawed at 0°C for 5h. Fish were packed – two fillets per bag. Airtight bags with two fillets was then placed into the vacuum sealing machine, where the contents of the bag got sealed into an oxygen reduced environment. The next step was sous-vide cooking performed in a water bath. Different time – temperature treatments were applied during sous – vide cooking. After cooking the fish was stored at chill temperature. Fillets were cooked in water baths (Grant, UK) at 60°C, 75°C and 90°C for 10, 15 and 20 min and immediately placed on ice and transported to a cold room, where it was stored for 1, 3 and 7 days at 0±1°C till analysis.

2.3. Extraction of lipids

A modified Bligh and Dyer method (Dyer 1959) was used to extract neutral lipids, polar lipids and free fatty acids. The extraction was done in duplicate. Lipids were extracted with: chloroform and methanol. Approximately 10g of minced fish was placed in each centrifugation bottle and kept on ice during the procedure. 16 ml of water, 40 ml of methanol and 20 ml of chloroform were added, and samples were homogenized for 2 minutes with an Ultra-Turrax. Further, 20 ml of chloroform was added and 20 ml of distilled water with 40 seconds homogenization after every addition. The weight of centrifuge bottles was adjusted with distilled water before centrifugation at 2600 g for 15 minutes. The samples were collected as follow:

- 2 x 2 ml of methanol/water phase for TBARS in methanol/water phase analysis
- 2 x 200 µl of chloroform phase for TBARS in chloroform phase analysis
- 2 x 1 ml of chloroform phase for PV analysis
- 2 x 2 ml of chloroform phase for determination of total lipid content

Collected samples were covered with nitrogen and stored in the freezer till the day of analyzer.

2.4. Compositional analysis

The total lipid content was determined gravimetrically. 2 ml of chloroform phase sample was pipetted out into previously weighed kimax tube and the chloroform was evaporized on heating block at 60°C under a flow of nitrogen gas. Further, the sample was cooled down and

kept overnight in a desiccator, weighed next day and the percentage of total lipid content was calculated by the use of equation 2.1:

$$\%\ \text{Total lipids} = \frac{(\text{weight tube and evaporated sample (g)} - \text{weight tube (g)}) * 100}{\text{weight sample (g)}} * 20\ \text{ml chloroform} \qquad (2.1)$$

The results were expressed in percentage of total lipid content of mackerel fillets.

2.5. Quality analysis

2.5.1. TBARS in chloroform phase

Determination of TBARS in chloroform phase was performed in duplicate according to Ke and Woyewoda (1979). 200 µl of extract was pipetted into kimax tube. Further, TBA working solution (containing thiobarbituric acid, chloroform, sodium sulphate and butylated hydroxytoluene) was added and mixed with a vortex for 15 seconds. After mixing tubes were incubated in a boiling water bath for 45 minutes and cooled down. 2,5 ml of TCA solution containing trichloracetic acid was added, tubes were inverted and centrifuged for 10 minutes at 900g.

The standard curve was prepared in duplicate in the same manner as samples, with the exception of replacing the sample with TEP solution in following concentrations: 0, 25, 50, 100, 150 and 200 µl.

The absorbance was measured at 538 nm in a plate reader spectrophotometer (TECAN, Infinite M200 PRO, Austria). To calculate the TBARS value, the followed equation (2.2) was used:

$$\mu\text{Mol TBARS/g lipid} = \frac{(\text{absorbance of sample} - \text{intercept of standard curve})}{(\text{slope of standard curve} * \text{total lipid content in sample} * 1000} \qquad (2.2)$$

where total lipid content is given as µl / g lipid, and 1000 is the conversion factor to get µmoles/g.

2.5.2. TBARS in methanol/water phase

Determination of TBARS in methanol/water phase was performed in duplicate, according to Schmedes and Holmer method from 1989. 2 ml of methanol/water from lipid extraction was pipetted out to kimax tube. Further, 2 ml of TBA reagent containing thiobarbituric acid and trichloracetic acid was added, sample was mixed with a vortex mixer for 15 seconds. Samples were incubated in boiling water for 30 minutes, cooled down and centrifuged at 300gt for 10 minutes. The absorbance was measured at 532 nm in a plate reader spectrophotometer (TECAN, Infinite M200 PRO, Austria). To calculate the TBARS value equation (2.3) was used:

$$\text{nmol TBARS/g muscle} = \frac{(\text{absorbance of sample} + \text{intercept of standard curve}) * 76\text{ml}}{\text{slope of standard curve} * 2\text{ml} * \text{wet weight of sample (g)}} \quad (2.3)$$

Where 76 ml is the methanol and water added during the extraction and 2 ml is the total amount added during the TBARS procedure.

The standard curve was prepared in duplicate. TEP solutions in the following concentrations: 0, 25, 50, 100, 150 and 200 µl were pipetted into kimax tubes. Next, 2 ml of the solution of methanol/water (1:0,9) was added, in addition to TBA working solution containing tiobarbituric acid. The standards were treated in the same manner as the samples.

2.5.3. Peroxide value (PV)

The PV value was determined in duplicate for each extract. 1 ml of extract was pipetted out into a kimax tube. 10 ml of chloroform/methanol mix was added, covered with nitrogen and mixed. Next, 50 µl of thiocyanate solution and 50 µl of Fe(II)chloride - solution was added. Samples were incubated for 5 minutes in darkness, at room temperature. Additionally, two reagent blank samples were prepared (contained everything except the extract samples) and 2 Fe(III) standard samples, where Fe(III)-standard solution was used instead of lipid extracts. The standard curve was prepared in duplicate by dilution the FE(III) stock solution in 100 m volumetric flasks with chloroform/methanol according to table 2.2:

Table 2.2: Standard range for PV standard curve.

	µl stock-solution in 100 ml volum. flask	Concentration of Fe (III) [µg Fe(III)/10 ml][3]
1	0	0
2	50	1,03
3	250	5,16
4	500	10,33
5	1000	20,66
6	1500	30,99
7	2000	41,32

Thiocyanate solution was added, and standards were incubated together with the samples. The absorbance was measured at 500 nm in a plate reader spectrophotometer (TECAN, Infinite M200 PRO, Austria). To calculate the PV value equation 2.4 was used:

$$V = \frac{(A_s - A_b) * k}{55{,}8 * m * 2} \text{meq}O_2/\text{kg oil} \qquad (2.4)$$

Where:

A_s is the absorbance of the sample at 500 nm;

A_b is the absorbance of reagent blank at 500 nm;

k – value is the slope of standard curve;

55,85 is the molecular weight of Fe;

2 is the conversion factor from meq oxygen to meq peroxide

m is the weight of oil in the extract;

$$m = \frac{\text{g extract for the determination of PV} * (\text{glass with oil} - \text{empty glass})}{\text{g extract for the determination of oil content}} \qquad (2.5)$$

For sous-vide samples PV was quantified by the iodometric titration method described in AOCS official methods (Cd 8b-90). Automatic titrator (TitroLine 7800, Xylem Analytics, Germany) coupled with a Platinum electrode was used to determine the end point of titration. The analysis was performed in duplicate and the result were expressed in $\text{meq}O_2/\text{kg}$ oil.

2.5.4. Color parameters

Color parameters were measured in color coordinates: L* - (lightness, black = 0, white =100), a* - (redness >0, greenness <0), and b* - (yellowness, b* >0, blue <0) according to the Commission Internationale de l'Éclairage (CIE) Lab scale. Data were measured by a Minolta Chroma meter CR-400 (Konica-Minolta, Osaka, Japan), which was calibrated with a standard white plate. Three readings were taken from each sample and the average was calculated.

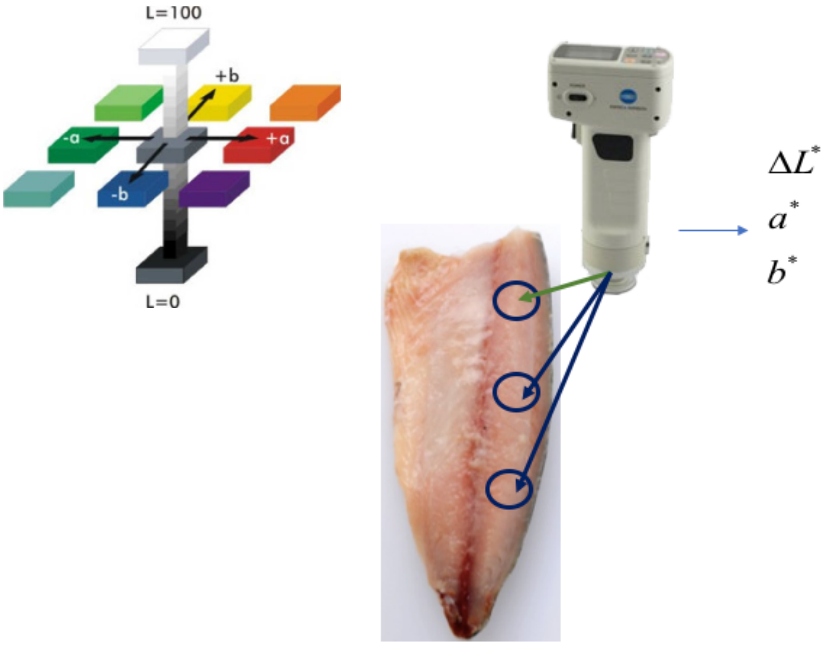

Figure 2.2: Color measurements.

Total color difference was calculated according to the equation 2.6:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2.6)$$

where L_0, a_0 and b_0 are values of the control sample.

2.6. Chemicals and equipment

Chemicals used for different analysis are listed in table 2.3 below, while equipment are listed in table 2.4. Each table contain name of the chemical/equipment, method for which it was used and name of the producer.

Table 2.3: Chemicals used for analysis; name, method for which they were used, and producer names are included.

Chemical	Analyses	Producer
2-thiobarbituric acid (TBA)	TBARS	Sigma-Aldrich
Acetic acid, glacial	TBARS	Merck
Ethanol	TBARS, PV	Merck
Chloroform ($CHCl_3$)	TBARS, lipid extraction, PV	Merck
Butylated hydroxytoluene (BHT)	TBARS	Sigma-Aldrich
Trichloracetic acid (TCA)	TBARS	Merck
Water, distilled	TBARS	NTNU
Methanol (CH_3OH)	TBARS, lipid extraction, PV	Merck
Fe(III)- stock solution	PV	SINTEF
Hydrochloric acid (HCl) 37%	PV	Merck
Ammonium thiocyanate	PV	Merck
Barium-dichloride ($BaCl_2$ *2 H_2O)	PV	Sigma-Aldrich
Iron (II)- sulphate heptahydrate ($FeSO_4$ * 7 H_2O)	PV	Merck
1,1,3,3- tetraethoxypropane (TEP)	TBARS	SINTEF
Sodium Sulphite (Na_2SO_3)	TBARS	Merck
Nitrogen (N_2) (gas)	PV	

Table 2.4: Equipment used for analysis; name, method for which they were used, and producer names are included.

Equipment	Analyses	Producer
Vortex-mixer	TBARS, PV	MS2 Minishaker IKA
Plate reader spectrophotometer	TBARS, PV	TECAN, Infinite M200 PRO, Austria
Centrifuge for kimax tubes	TBARS, PV	Allegra X-15R Centrifuge, Beckman Coulter
Centrifuge	Lipid extraction	Sigma
Evaporating unit	PV, total lipid content	Pierce, Reacti-Vap
Homogenizer	Lipid extraction	Ultra-Turrax
Laboratory scale	TBARS, lipid extraction, PV	AG204 Delta Range, Mettler Toledo
Magnet stirrer	TBARS, PV	Heidolph MR 3001K
Vacuum sealing machine	Sous - vide	Webomatic Vacuum packaging system, Super max, 3000 sensor
Water baths	Sous - vide	Grant, UK
Automatic titrator	Sous-vide - PV	TitroLine 7800, Xylem Aalytics, Germany
Minolta Chroma meter CR-400	Color parameters	Konica-Minolta, Osaka, Japan

3. Results

The main objective of this work was to study lipid oxidation during chilled, superchilled and frozen storage, and after sous – vide cooking of fillets of Atlantic mackerel (*Scomber scorbus*). Knowledge about the oxidative stability in mackerel lipids during different types of storage can be important for industry.

All results are presented as comparative charts and include Standard Deviation (SD) as calculation of deviation. The raw data are presented in the appendix section.

3.1. Cold storage

3.1.1. Total lipid content

No significant difference was found in the total lipid content. Figure 3.1 shows variations in total lipid content within type and duration of storage. The average lipid content in chilled samples varied between 21,71% and 22,62%, superchilled 19,71% to 22,42% and for frozen fish it was in the range of 20,42% to 22,68%. Small or no variations indicate that type and duration of storage have no significant influence on total lipid content. Measurement data is given in appendix A.

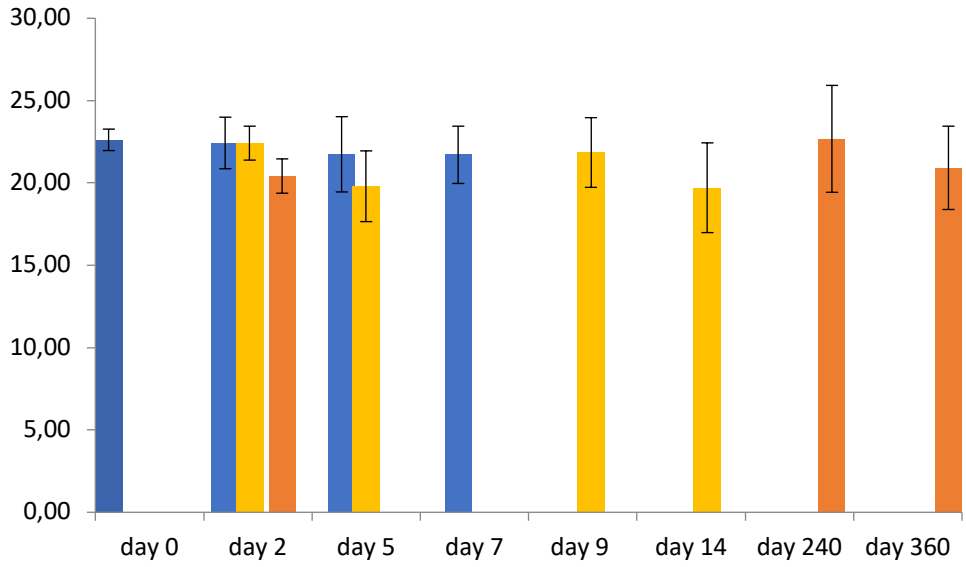

Figure 3.1: Total lipid content *(mean ± SD) in % in chilled mackerel after 0, 2, 5 and 7 days of storage; chilled mackerel after 2, 5, 9 and 14 days of storage and frozen mackerel after 2 days, 8 months and one year of storage. Each column represents an average of 6 samples.*

3.1.2. Primary oxidation products

3.1.2.1. PV

Figure 3.2 presents the primary oxidation products measured as peroxide value (PV). Varying levels of PV are found between the different types of storage (chilling, superchilling and freezing). For chilled samples PV increased with time from 3,04 meq O_2/kg oil in average at day 0 to 5,82 meq O_2/kg oil in average at day 7. Significantly higher PV is observed for superchilled samples at day 5 and 9, 6,43 and 5,80 meq O_2/kg oil respectively. Frozen samples are characterized by the lowest PV values (1,56 meq O_2/kg oil to 4,17 meq O_2/kg oil). In superchilled and frozen samples PV decrease with time. The PV level changes from time and type of storage. None of the samples exceeded 10 meq O_2/kg oil. Some high standard deviations are obtained for superchilled samples. It is important for the industry to know how different types of storage influence raw material without antioxidants. Storage conditions influence the rate of lipid oxidation and thus the PV. Therefore, the course of increasing of PV is examined. Measurement data is given in appendix B.

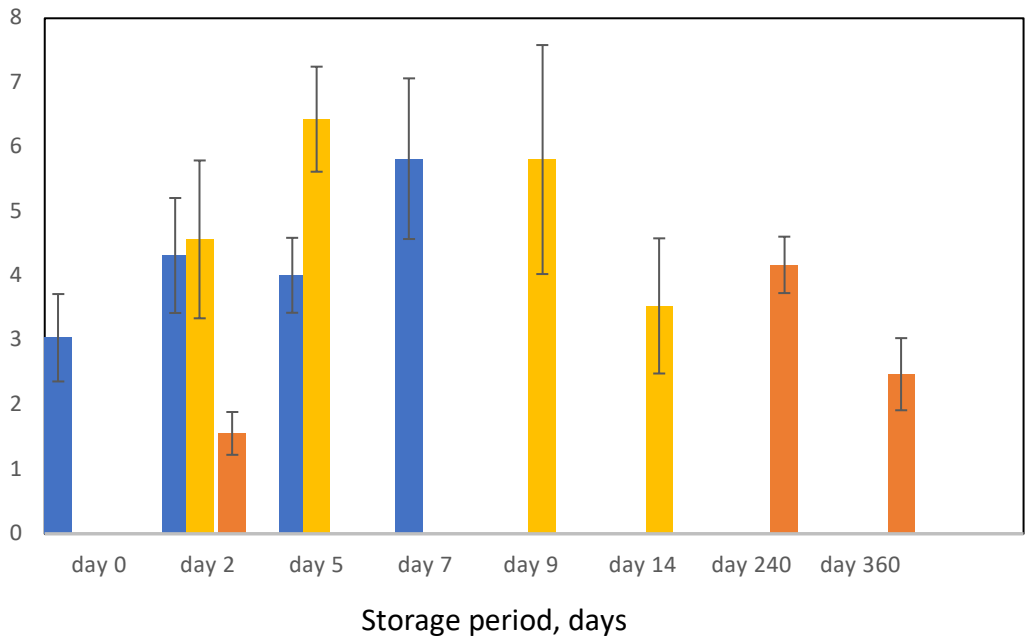

Figure 3.2: Peroxide value (PV) (means ± SD) in meq O2/ kg oil determined in chilled mackerel after 0, 2, 5 and 7 days of storage, superchilled mackerel after 2, 5, 9 and 14 days of storage and frozen mackerel after 2 days, 8 months and one year. Each column represents an average of 6 samples.

3.1.3. Secondary oxidation products

In order to determine the formation of secondary oxidation products, analysis of TBARS (thiobarbituric acid reactive substances) both in the methanol/water and chloroform phase were performed. TBARS mainly represents the level of aldehydes in the oxidized lipids.

3.1.3.1. TBARS in chloroform phase

Figure 3.3 represents the results of TBARS determined in chloroform phase expressed as µmol TBARS/g lipid. Varying levels of TBARS have been found depending on type of storage and storage duration. TBARS for chilled samples increased gradually with time from 0,51 µmol TBARS/g lipid to 1,13 µmol TBARS/g lipid. Secondary oxidation has developed in a greater extent in superchilled samples during the first five days of storage after which it is drastically reduced. Frozen samples are characterized by low level of TBARS (0,15 µmol TBARS/g lipid to 0,70 µmol TBARS/g lipid). The most significant difference can be observed between superchilled and frozen storage. For the measurement of TBARS after superchilled storage, relatively large standard deviations were obtained, whereas for chilled and frozen samples standard deviations are rather small. Experimental data is given in appendix D.

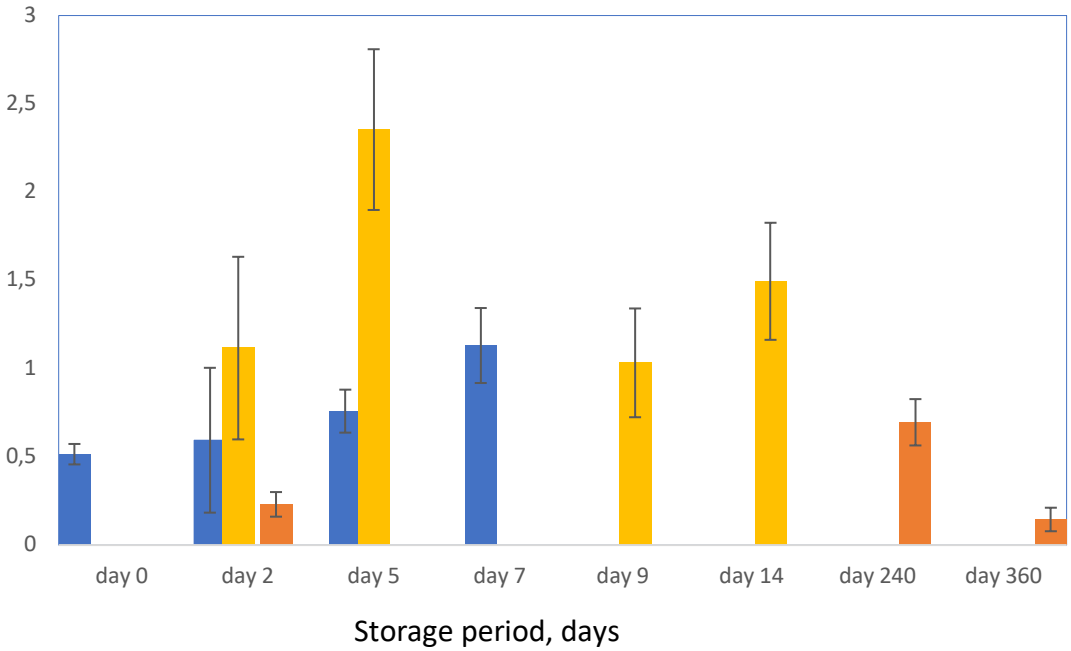

Figure 3.3: TBARS in chloroform phase (means ± SD) in µmol TBARS/g lipid determined in chilled mackerel after 0, 2, 5 and 7 days of storage, superchilled mackerel after 2, 5, 9 and 14 days of storage and frozen mackerel after 2 days, 8 months and one year. Each column represents an average of six samples.

3.1.3.2. TBARS in methanol/water phase

TBARS in methanol/water phase are shown in figure 3.4 and are given in nmol TBARS/g lipid. TBARS in chilled fish slightly decreased with time and are significantly lower than in other types of storage. In frozen samples increase of TBARS can be observed. For superchilled samples TBARS are relatively constant and marginally higher than for chilled and frozen samples. Measurement data is given in appendix C.

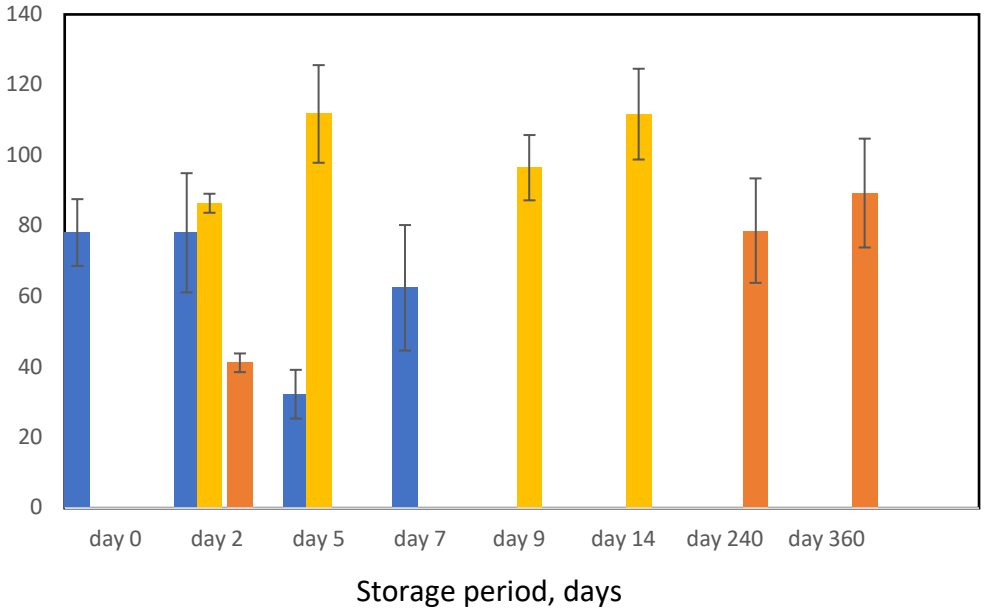

Figure 3.4: TBARS in methanol/water phase (means ± SD) in nmol TBARS/g lipid determined in chilled mackerel after 0, 2, 5 and 7 days of storage, superchilled mackerel after 2, 5, 9 and 14 days of storage and frozen mackerel after 2 days, 8 months and one year. Each column represents an average of 6 samples.

3.1.3.3. Color parameters of chilled and superchilled mackerel samples

Graph 3.5, 3.6 and 3.7 represents color measurements for chilled, superchilled and frozen storage, respectively. For chilled fish the lightness gradually increased during the storage, for superchilled fish it increased during the first two days of storage and after remained constant. For frozen samples there was no changes in lightness during storage. Yellowness tends to increase for chilled and frozen mackerel, while for superchilled the values vary between different days of storage. Redness displays no pattern for chilled and superchilled samples, while for frozen the values remain constant.

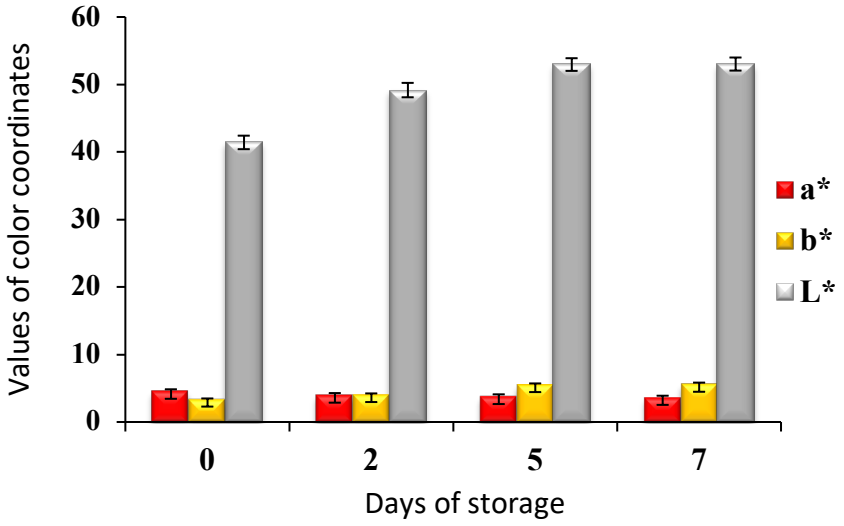

Figure 3.5: Color characteristics of chilled mackerel fillets, at 0, 2,5 and 7 days of storage.

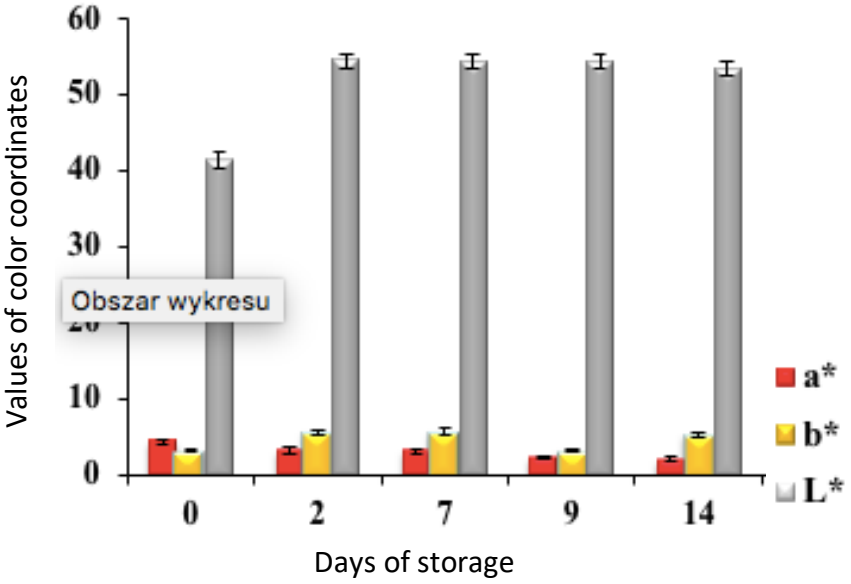

Figure 3.6: Color characteristics of superchilled mackerel fillets, at 0, 2, 7, 9 and 14 days of storage.

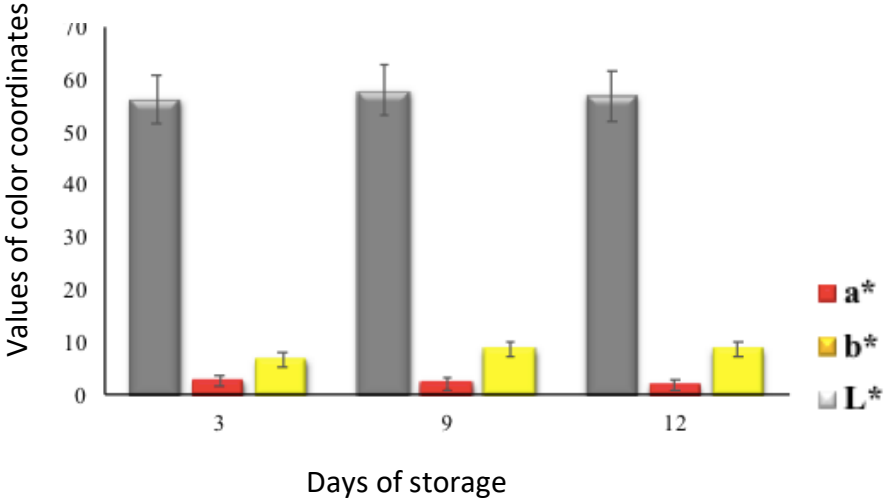

Figure 3.7: Color characteristics of frozen mackerel fillets.

A significant correlation between the secondary products of lipid oxidation, expressed as TBARS and yellowness of the fish tissue (b*- value) for chilled, superchilled and frozen mackerel was found.

Chart 3.8 presents total color difference for chilled and superchilled mackerel samples. Calculated total color differences for chilled and superchilled samples show that for chilled mackerel fillets total color difference increase with duration of storage, while for superchilled mackerel ΔE slightly decrease over time.

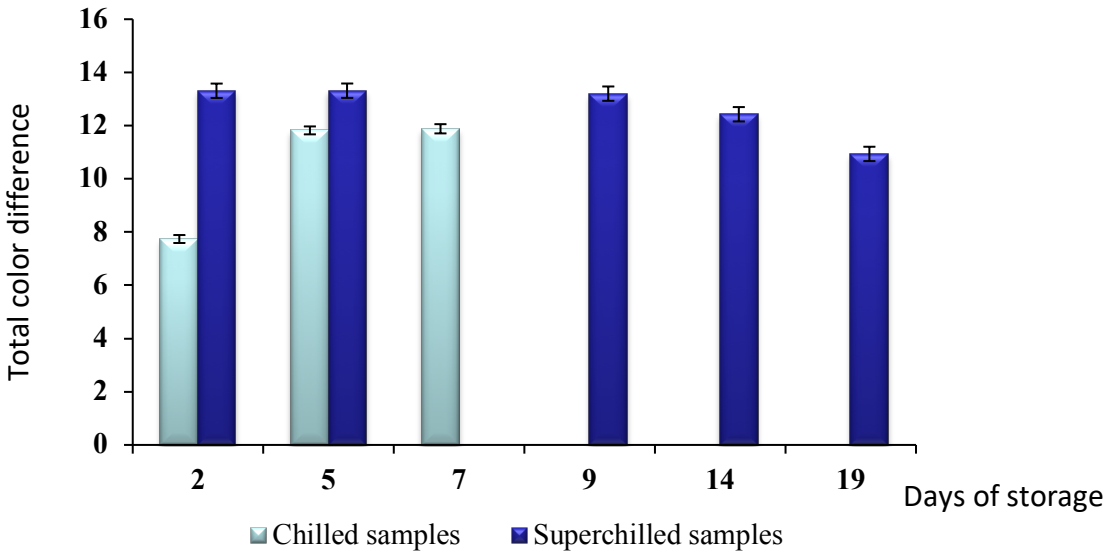

Figure 3.8: Total color difference (ΔE).

3.2. Sous- vide

3.2.1. Primary oxidation products

3.2.1.1. Peroxide value (PV)

PV was determined by the iodometric titration method. Peroxide values which represents the primary oxidation products are shown in figure 3.9. The initial PV of raw mackerel fillets was 3,15±0,06 meq O2/kg oil and PV increased significantly after sous-vide treatment and during further chilled storage. In most cases PV exceeded the acceptable level of 10 meqO2/kg oil. Samples cooked at 60ºC and 75ºC for 10 and 15 minutes and stored for one day had lower PV than the other samples. Larger increase in PV was observed for samples cooked at 90ºC for 15 and 20 minutes than for the other samples. Temperature and time of sous-vide cooking did not have large influence on formation of primary oxidation products, however the duration of chilled storage after cooking lead to a significant, gradual increase in peroxide value of mackerel (from 5,73 to 24,20 meqO2/kg oil).

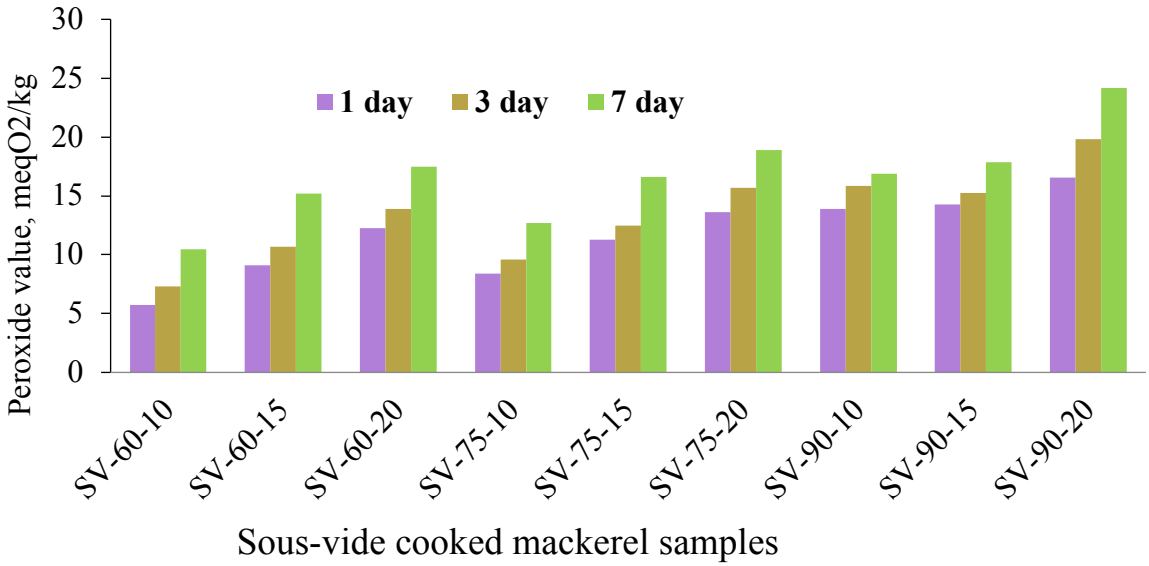

Figure 3.9: Peroxide value (PV) in meq O2/ kg oil determined in sous-vide mackerel after 1,3 and 7 days of chilled storage. The codes for samples are Sous-vide(SV)-temperature of cooking-duration of cooking.

3.2.2. Secondary oxidation products

3.2.2.1. TBARS

Figure 3.10 represents the secondary oxidation products measured in sous-vide cooked mackerel fillets. The initial TBARS value of raw mackerel fillets was 0,61±0,22 mmol MDA/kg. During chilled storage TBARS increased, reaching the highest level (5,23 mmol MDA/kg) for fillets cooked for 20 minutes at 90ºC and stored at chilled temperature for 7 days. The highest degree of oxidation was obtained for samples cooked for 15 and 20 minutes and stored for 7 days. There are significant differences between analyzed mackerel samples, which means that increase in cooking temperature, time of cooking and duration of chilled storage lead to a significant increase in content of secondary oxidation products.

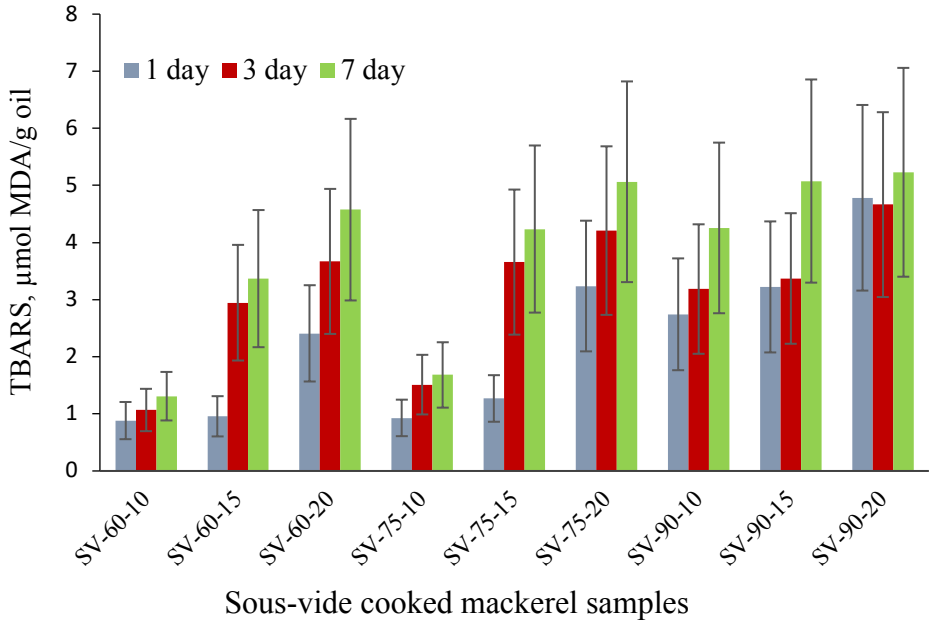

Figure 3.10: TBARS expressed as µmol MDA/g oil, determined in sous-vide mackerel after 1,3 and 7 days of chilled storage. The codes for samples are Sous-vide(SV)-temperature of cooking-duration of cooking.

3.2.2.2. Color measurements

Color measurements were performed on selected locations at the surface of mackerel fillets. Three readings were taken, and the average was calculated in order to calculate color parameters: L* - (lightness, black = 0, white =100), a* - (redness >0, greenness <0), and b* - (yellowness, b* >0, blue <0). The color parameters of control sample were: L* = 59,18±3,89; a* = 2,46±0,69 and b* = 8,48±1,14.

L* - (lightness, black = 0, white =100)

Lightness displayed a significant increase during chilled storage compared to the lightness of initial raw mackerel samples (L* = 59,18±3,89) (Figure 3.11).

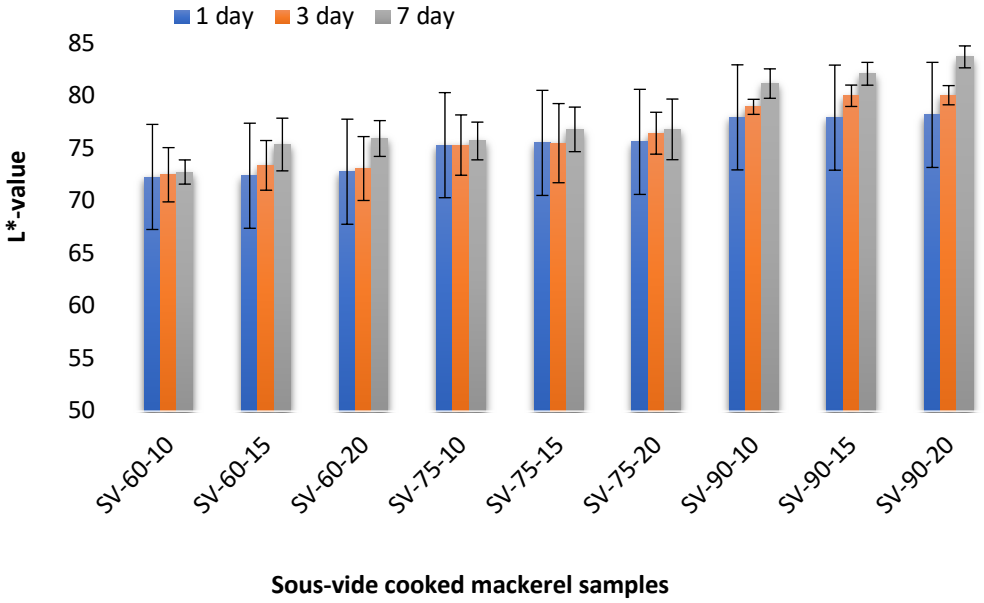

Figure 3.11: Color measurements; L - (lightness, black = 0, white =100) according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Three readings were taken from each mackerel fillet. Color parameters of control sample was 59,18±3,89.*

a* - (redness >0, greenness <0)

There are large variations of a* values and no pattern can be seen for sous-vide mackerel. Mackerel fillets cooked at 60ºC tend to decrease in redness during chilled storage time due to myoglobin denaturation, while fillets subjected to heat treatment at 90ºC gradually increase in redness over the chilled storage. The reason for increased redness is most likely formation of carboxy heme pigment in vacuum packed fish. No pattern in development of redness in the flesh of mackerel samples exposed to heat treatment at 75ºC was observed.

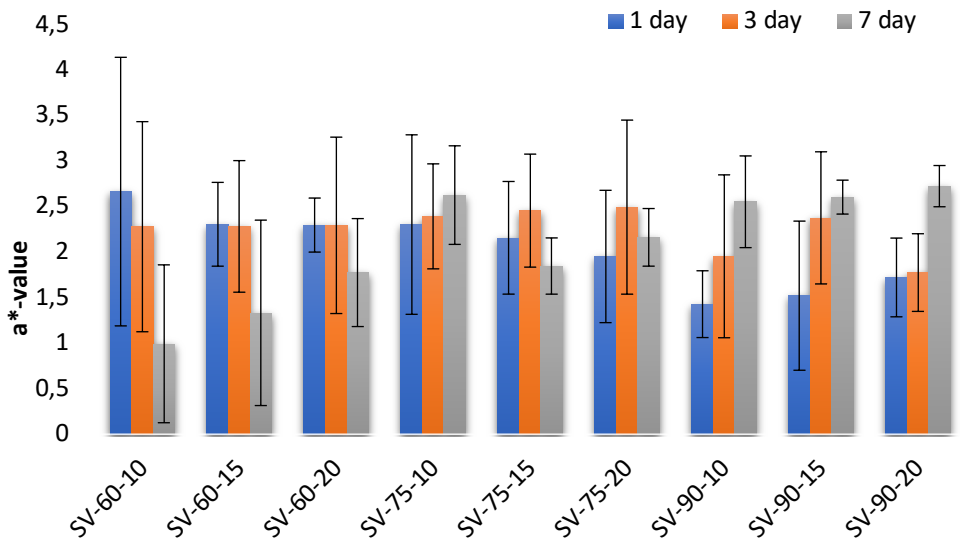

Figure 3.12: Color measurements; a - (redness >0, greenness <0) according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Three readings were taken from each mackerel fillet. Color parameters of control sample was a* = 2,46±0,69.*

b* - (yellowness, b* >0, blue <0)

Yellowness displayed a significant increase during chilled storage in comparison to the color parameters of initial raw mackerel samples (b* = 8,48±1,14). Yellow, off-color formation can be observed under the skin of mackerel fillet after sous-vide treatment and chilled storage as presented in Figure 3.13.

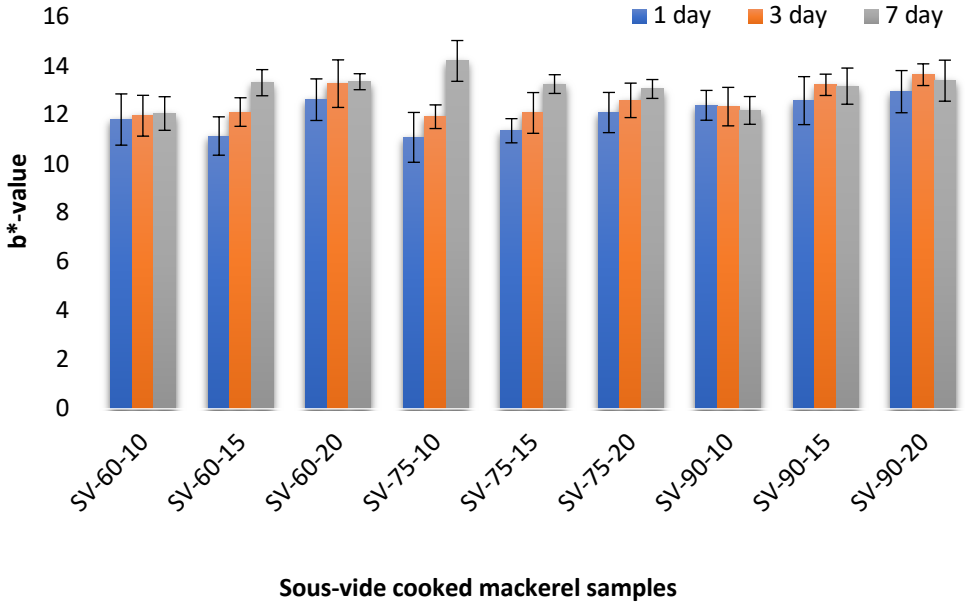

Figure 3.13: Color measurements; b - (yellowness, b* >0, blue <0) according to the Commission Internationale de l'Èclairage (CIE) Lab scale. Three readings were taken from each mackerel fillet. Color parameters of control sample was b* = 8,48±1,14.*

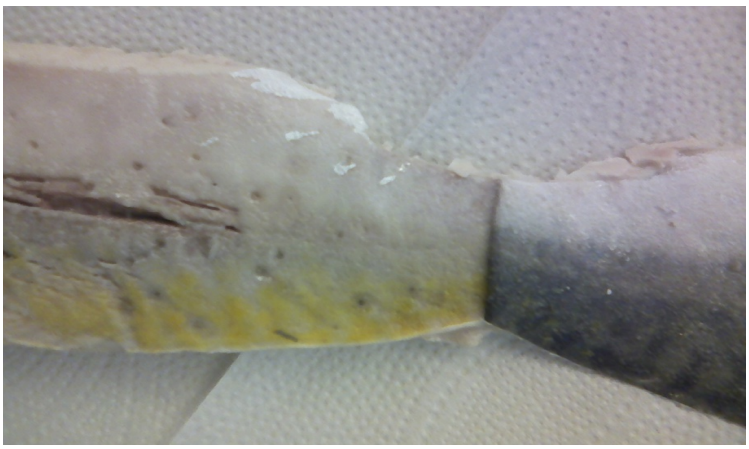

Figure 3.14: Picture of mackerel fillet after sous-vide treatment with observed color changes under the skin.

4. Summary

In general, higher values for PV, TBARS in chloroform and TBARS in methanol phase were obtained for superchilled samples compared to chilled and frozen fish, as described above.

Lipid content

Neither type or time of storage is found to influence the lipid content. The results show a relatively stable total lipid content for chilling, superchilling and freezing in range of 19,80% to 22,68%.

Primary oxidation

PV is found to change with storage time. For superchilled and frozen samples it can be observed that after some period of time PV starts to decrease. However, during chilled storage PV continue to increase with time. Type of storage also has influence on development of primary oxidation products. The highest PV was obtained in superchilled samples, while frozen fish was characterized by the lowest PV. The results obtained from measurements of primary oxidation products for sous – vide cooked fish, displayed a significant increase in PV after sous-vide treatment and chilled storage. The peroxide value was gradually increasing during chilled storage for all the samples and most of them exceeded the acceptable level (10 meqO2/kg oil). The primary oxidation products were increasing with duration of chilled storage. Exposure to higher temperatures and longer times of cooking accelerate lipid oxidation. It shows that sous – vide treatment leads to increased lipid oxidation.

Secondary oxidation

Values of TBARS both in chloroform phase and methanol/water phase for superchilling displays higher values than chilling and freezing. TBARS in the chloroform phase increases for chilled fish but in methanol/water phase slightly decreases over time due to breaking down secondary oxidation products into short chain products. Significant differences are found between superchilled (1,03 – 2,35 μmol TBARS/g lipid) and frozen samples (0,15 – 0,70 μmol TBARS/g lipid) for TBARS in chloroform phase, while differences in TBARS in methanol/water phase are relatively small. After sous – vide treatment TBARS progressively increased during the chilled storage, reaching the highest value for fillets cooked for 20 minutes at 90ºC, stored for 7 days. Higher temperature and exceeded time of cooking together with duration of chilled storage significantly increase TBARS content in mackerel.

Color measurements

Off-color of mackerel fillets are related to lipid oxidation and development of primary and secondary oxidation products. Accumulation of secondary oxidation products of lipid oxidation

contributed to changes in yellowness of sous-vide mackerel flesh during chilled storage, thus yellowness displayed a significant increase during chilled storage. The off-color changes can be observed on mackerel flesh under the skin. Lightness also tend to increase with duration of chilled storage. Redness however, displays high variations and make the tendency vague.

5. Discussion

The overall aim of the project ProHealthPelagic is to increase the consumption of pelagic fish species, like Atlantic mackerel. Atlantic mackerel (*Scomber scombrus*) have many beneficial effects due to high amount of valuable lipids (such as omega – 3 fatty acids) and other bioactive compounds. However, because of high amount of myoglobin in dark muscles and also polyunsaturated fatty acids this fish is highly prone to lipid oxidation, leading to decrease in these health benefits, while decreasing sensory and nutritional profile. In order to protect Atlantic mackerel from lipid oxidation during storage, a number of preservation/mild processing methods were explored in this study.

Development of primary and secondary products of lipid oxidation in chilled superchilled, frozen and sous-vide cooked mackerel fillets was studied in order to examine oxidative stability.

Mackerel as fatty fish consist between 5% - 30% of total lipid content, which varies greatly depending on the season. The lipid content is usually higher in the winter half-year (about 25%) ant it is lower around the summer (about 15%) (Standal, Mozuraityte et al. 2018). The lipid content was stable during storage, with the average between 19,71% - 22,68%, this is in agreement with other results, where duration of frozen storage did not influence the lipid content in mackerel (Standal, Mozuraityte et al. 2018). The fish was caught at the same time and the same place, so there were no differences correlated with season, location of the catch, age etc. The slight changes in lipid content compared at different storage temperatures may come from individual variations.

The oxidative stability was determined by evaluation of primary (PV) and secondary (TBARS) oxidation products. Dulavik et al. have found a slight increase in TBARS in the frozen fillets of saithe. They have made a conclusion that relatively high fat content and the amount of pro-oxidant metals contributes to high susceptibility of lipids in the dark muscle to oxidation of lipids. Dark muscle contain larger amount of metals what contribute to the higher content of myoglobin and hemoglobin (Dulavik 2007). When fish is processed lipids derived from tissues are exposed to pro-oxidants (oxygen, enzymes, metals, etc.). Trace amounts of redox active metals such as iron (Fe) and copper (Cu) can enhance lipid oxidation acting as promoters. In the presence of hydroperoxides they generate free radicals (Maestre, Pazos et al. 2011, Wąsowicz et al. 2004). Transition metal ions are involved in hydroperoxide decomposition. Metals are considered to be the main promoters of autoxidation, mainly because

it is difficult to eliminate them during food processing and they can be derived from plant and animal tissues.

PV value highly depends on processing conditions, as well as presence of antioxidants, which were not used in this study (Turner, McLean et al. 2006, Wąsowicz et al. 2004). Free radicals generated during hydroperoxide (metal-catalyzed, photo-catalyzed or thermal) decomposition are initiators of autoxidation. Radicals formed from blood, transition metals etc. also enhance the oxidation. Photo-oxidation can be initiated by pigments present in food, such as porphyrins (hemoglobin, myoglobin). Hemoglobin and myoglobin are very strong prooxidants, stronger than iron alone (Wąsowicz et al. 2004). All these factors could accelerate the lipid oxidation because as it was mentioned earlier, no antioxidants were used in this study.

Standal et al. have found that during chilled and frozen storage of skin-on mackerel fillets there was no significant changes in primary oxidation products measured as conjugated dienes (CD), whereas Saeed and Howell (2002) and Aas et al. (2013) observed small increase in PV and TBARS in minced Atlantic mackerel fillets stored at -30°C and intact mackerel fillets stored at -30°C respectively. They assumed that the mincing operation could have been the reason for reduced lipid stability compared to intact fillets (Standal, Mozuraityte et al. 2018, Saaed and Howell 2002). The mincing operation could also affect lipid stability in case of this research.

Peroxide value varied between samples kept in different storage temperatures, however none of them exceeded recommended 10 meqO2/kg oil. The highest PV value was obtained for superchilled samples at day 5, while frozen samples had relatively low PV. According to Icekson et al. (1998) relatively low and stable PV during frozen storage can indicate that the rate of primary oxidation products formation is equal to the degradation rate. Products of primary lipid oxidation contribute to the formation of secondary lipid oxidation products (Icekson et al. 1998). The results from this study confirm this hypothesis. The increase in PV is influenced by content of prooxidants and large amount of myoglobin in dark muscle. The results from superchilled samples revealed values significantly higher for peroxide value and TBARS in chloroform phase and slightly higher for TBARS in methanol and water phase compared with chilling and freezing.

Schmedes and Hølmer proved that MDA is also present in the methanol/water phase, together with amino acids and peptides which are present to some extent. The MDA present in the methanol/water phase is already formed from hydroperoxide cleavage and the second release occurs due to the heating step (Schmedes 1989). Moreover, some fractions of lipids are

also present in this phase. Therefore, the TBA test in methanol/water phase was also performed as addition to analysis of TBARS in chloroform phase where most of the lipids were extracted.

TBARS were significantly higher for superchilled fillets than for chilled and frozen fish. Frozen samples had relatively low TBARS values, which decreased with storage time. Similar results were obtained by Sandal et al., where TBARS values were relatively constant and slightly decreased after one-year storage and by Icekson et al. where TBARS values decreased dramatically after 12 months of frozen storage. The explanation for this can be found in further reactions of carbonyls, for example with proteins forming Schiff bases (Standal, Mozuraityte et al. 2018, Icekson et al. 1998). For superchilled and frozen samples a relationship between TBARS in chloroform and TBARS in methanol/water phase can be observed. Both TBARS show similar pattern of increase. Rapid decrease in PV correlates with formation of secondary oxidation products. The formation of secondary oxidation products starts later and at lower rate than development of PV. Storage at chilled and superchilled temperature decreased the induction period and resulted in higher and faster formation of TBARS than for frozen fish. However, because of high reactivity of TBA, there is a possibility that extraction and analysis method influenced TBARS formation. The color measurements revealed the contribution of accumulation of secondary oxidation products to yellowness of fish flesh. These findings are in agreement with other studies (Cropotova et al. 20XX) on the effects of cold storage on lipid oxidation.

Even though fresh fish is usually chosen over frozen, preservation of mackerel fillets by freezing seems to be a better option than prolonged chilled storage. Superchilling can be a good alternative for chilled storage, however the use of antioxidants should be considered and further studied to ensure the high quality of the product.

Presented studies also demonstrated the influence of different sous-vide regimes and further chilled storage on properties of lipid of Atlantic mackerel. Peroxide value displayed a significant, gradual increase for all sous-vide cooking and chilled storage samples. Obtained peroxide values were considered as high because most of the exceeded recommended value 10 meqO2/kg oil. According to other studies TBARS slightly increase during refrigeration storage of salmon fillets after sous – vide cooking (Diaz, Garrido et al. 2011). Higher temperatures and longer times of sous-vide cooking resulted in increase in PV. TBARS, which contribute to off-flavor and rancid odors were progressively increasing over time of chilled storage. This leads to the conclusion that the strongest effect on formation of primary and secondary lipid oxidation products in mackerel is the duration of chilled storage. Increase in temperature and time of sous-vide cooking also increased TBARS content in mackerel, probably due to thermal breakdown

of primary oxidation products. However, increase in temperature and time of sous-vide cooking had the lowest contribution to lipid oxidation and color changes.

Evaluation of color parameters revealed that the main effect on lightness and yellowness of mackerel is attributed to duration of chilled storage because those instrumental color parameters were significantly affected by chilled storage time. A significant increase in yellowness was a consequence of duration of sous-vide cooking and/or storage time. The increase in yellowness of analyzed samples during the storage was probably due to accumulation of compounds generated by decomposition of primary and secondary oxidation products. Values of PV and TBARS were positively correlated with yellowness what can indicate that mackerel samples had a higher lipid peroxidation level than raw mackerel. Because duration of chilled storage exhibits the highest significant effect on changes in sous-vide cooked mackerel and contribute greatly to the formation of primary and secondary products of lipid oxidation it was concluded that prolonged chilled storage of sous-vide cooked mackerel negatively influences lipid stability. Therefore, the addition of antioxidants in order control/minimize the oxidation process should be studied.

6. Conclusion

In conclusion, this study showed that different types of storage preserved the lipid from oxidation at different levels. Both, primary and secondary oxidation products formation increased with storage duration. It is clear that time is influencing the development of lipid oxidation products. Even though PV values tend to increase, they were well under the recommended limits for human consumption for chilled, superchilled and frozen storage.

The storage conditions (type of storage and storage duration) did not significantly influence the total lipid content of the mackerel fillets. There were no changes in total lipid content with different storage methods. Total lipid content was stable during storage, with the average 19, 71% - 22,68%.

During chilled storage slow, progressive lipid deterioration was observed over time. Yellowness development also increased over time which indicates the accumulation of secondary oxidation products.

Lipid oxidation took place especially between second and fifth day of superchilled storage, where rapid increase in PV and TBARS in chloroform phase was observed. TBARS in methanol and water phase were characterized by small variations. PV and TBARS in chloroform phase were significantly higher than for chilled and frozen fish. Yellowness increased during first two days of storage. However, no obvious tendency was found in formation of oxidation products for superchilled storage, indicating that the oxidative stability of lipid can occur differently. In conclusion, further studies are needed to improve superchilling. Antioxidants should be considered as additives. In order to retain good quality of the fish during superchilling the process should be improved to minimize lipid oxidation. Pelagic fish as highly perishable food is usually preserved by frozen storage, while superchilling can be an effective alternative.

Presented study indicated limited lipid oxidation rate by frozen storage of mackerel fillets. The values for PV and both TBARS were lower than for other type of storage. PV and TBARS in chloroform phase increased over first eight months and after that started to decrease. No color changes were observed. Preservation of mackerel fillets by freezing seems to be a better option compared to prolong chilled and superchilled storage and the quality of lipids was well preserved up to 12 months of frozen storage.

After sous – vide cooking increase in both, storage duration and temperature of cooking resulted in increased oxidation. Moreover, prolonged storage of the mackerel fillets had negative effect on quality. All of those factors resulted in gradual increase of PV and most of

the samples exceeded recommended 10 meqO2/kg. TBARS also tend to increase together with duration and temperature of cooking, and during chilled storage. Off - color development was attributed to duration of chilled storage and time of sous – vide cooking of mackerel fillets. This indicates that the formation and accumulation of secondary oxidation products affect sensorial properties. It can be concluded that sous – vide cooked fish lead to progressive loss of lipid quality during prolonged storage.

7. References

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8. Appendix

8.1. Appendix A. Total lipid content

Appendix A contains experimental data used for determination of total lipid content in chilled mackerel stored for 0, 2, 5 and 7 days; superchilled mackerel stored for 2, 5, 9 and 14 days; and frozen mackerel stored for 2 days, 8 months and one year. The results are calculated by using the equation 2.1. The raw data and calculated percentage of total lipid content are presented in Table 1.A, 2.A and 3.A.

Table 3.A: Data from determination of total lipid content given in percentage and standard deviation in chilled mackerel stored for 0, 2, 5 and 7 days.

day	sample	Fish weight (g)	Weight tube (g)	Weight tube + sample (g)	Total lipid content (%)	Average sample (%)	Total average (%)	Standard deviation
0 (25.01. 2017)	K031	10,08	10,7293	10,8388	21,73	22,00	22,62	0,65238966
	K032	10,09	10,7487	10,8611	22,28			
	K141	10,51	10,6978	10,8173	22,74	23,30		
	K142	10,2	10,8332	10,9549	23,86			
	K161	10,48	10,7272	10,8456	22,60	22,54		
	K162	10,28	10,9414	11,057	22,49			
2 (27.01. 2017)	K071	10,08	9,6181	9,7383	23,85	20,86	22,43	1,56696952
	K072	10,37	15,3068	15,3995	17,88			
	K101	10,05	9,1732	9,2974	24,72	23,99		
	K102	10,1	9,473	9,5905	23,27			
	K111	10,16	8,4605	8,5722	21,99	22,26		
	K112	10,26	11,5085	11,6241	22,53			
5 (30.01. 2017)	K061	10,48	10,8016	10,9019	19,14	19,2	21,74	2,28613765
	K062	9,73	11,9702	12,0631	19,10			
	K091	10,32	11,6754	11,7935	22,89	23,30		
	K092	10,46	10,8311	10,9551	23,71			
	K151	10,06	10,7792	10,8988	23,78	22,81		
	K152	10,17	10,8491	10,9602	21,85			
7 (01.02. 2017)	K171	10,5	8,8852	9,0033	22,50	22,73	21,71	1,74039808
	K172	10,6	9,4817	9,6034	22,96			
	K181	10,01	8,5377	8,6348	19,40	19,70		
	K182	10,48	8,5157	8,6205	20,00			
	K191	10,09	8,6788	8,7897	21,98	22,70		
	K192	10,12	8,8397	8,9582	23,42			

Table 4.A: Data from determination of total lipid content given in percentage and standard deviation in superchilled mackerel stored for 2, 5, 9 and 14 days.

day	sample	Fish weight (g)	Weight tube (g)	Weight tube + sample (g)	Total lipid content (%)	Average sample (%)	Total average (%)	Standard deviation
2 (27.01. 2017)	S011	10,56	8,55250	8,6755	23,30	23,46	22,42	1,029436
	S012	10,79	11,4363	11,5638	23,63			
	S041	10,1	8,4028	8,5135	21,92	22,37		
	S042	10,18	8,7278	8,844	22,83			
	S051	10,1	8,8196	8,9259	21,05	21,41		
	S052	10,32	8,5564	8,6687	21,76			
5 (30.01. 2017)	S071	10,1	8,4045	8,4917	17,27	17,32	19,80	2,14715245
	S072	10,31	11,4793	11,5689	17,38			
	S081	10,15	9,5375	9,6425	20,69	21,09		
	S082	10,24	9,5672	9,6772	21,48			
	S151	10,44	8,7721	8,8832	21,28	21,00		
	S152	10,11	9,2794	9,3841	20,71			
9 (03.02. 2017)	S061	10,2	8,7784	8,8761	19,16	19,36	21,85	2,15779753
	S062	10,25	8,6777	8,778	19,57			
	S101	10,14	10,0017	10,1176	22,86	23,22		
	S102	9,99	8,9289	9,0467	23,58			
	S141	10,27	9,5292	9,651	23,72	22,97		
	S142	10,38	8,7899	8,9052	22,22			
14 (08.02. 2017)	S021	10,02	9,8228	9,9319	21,78	22,15	19,71	2,72424644
	S022	10,28	8,7376	8,8534	22,53			
	S091	10,5	8,7571	8,8463	16,99	16,77		
	S092	10,4	8,6673	8,7534	16,56			
	S131	10,18	11,4656	11,5696	20,43	20,22		
	S132	10,22	9,0816	9,1838	20,00			

Table 5.A: Data from determination of total lipid content given in percentage and standard deviation in frozen mackerel stored for 2 days, 8 months and 1 year.

day	sample	Fish weight (g)	Weight tube (g)	Weight tube + sample (g)	Total lipid content (%)	Average sample (%)	Total average (%)	Standard deviation
2 (27.01. 2017)	F031	10,82	11,4356	11,5396	19,22	19,70	20,42	1,04091023
	F032	10,46	12,9795	13,085	20,17			
	F111	10,05	8,402	8,5011	19,72	19,95		
	F112	10,64	9,1934	9,3008	20,19			
	F131	10,33	9,8302	9,9436	21,96	21,62		
	F132	10,35	8,5505	8,6606	21,28			
8 months (27.09. 2017)	F021	10,43	10,8308	10,989	30,34	26,24	22,68	3,24693724
	F022	10,62	11,0173	11,1349	22,15			
	F091	10,69	11,0819	11,2002	22,13	21,92		
	F092	10,45	13,5135	13,6269	21,70			
	F101	10,32	10,3424	10,4504	20,93	19,88		
	F102	10,31	10,9722	11,0693	18,84			
1 year (19.01. 2018)	F011	10,12	10,9052	11,003	19,33	19,15	20,92	2,530312
	F012	10,07	10,8383	10,9338	18,97			
	F061	10,24	10,6306	10,7278	18,98	19,65		

	F062	10,21	10,8981	11,0018	20,31			
	F121	10,46	11,6759	11,7774	19,41	20,22		
	F122	10,03	10,7921	10,8976	21,04			
	F161	10,51	10,8383	10,9885	28,58	24,66		
	F162	10,12	11,0848	11,1897	20,73			

8.2. Appendix B. Peroxide value (PV)

Appendix B contains experimental data from determination of peroxide value in chilled mackerel stored for 0, 2, 5 and 7 days; superchilled mackerel stored for 2, 5, 9 and 14 days; and frozen mackerel stored for 2 days, 8 months and one year. The results are calculated by using the equation 2.4. The raw data and calculated peroxide value are presented in Table 9.B, 10.B and 11.B. PV is expressed as (meq O_2/kg oil). Standard curves used for calculations are presented in Figure 1.B, 2.B and 3.B, whereas data used for standard curve are presented in Tables 3.B – 8.B.

Table 1.B: Data used for standard curve for samples: K03, K06, K09, K14, K15, K16, S01, S02, S05, S09, S13, F03, F11, F13, where measurements were based on known concentrations of Fe(III). Obtained value of the slope was used for the calculations of peroxide value.

ul stock solution	Abs 500 nm	(ul Fe(III)/10ml)^3
0	0,0615	0
50	0,0742	1,03
250	0,167	5,16
500	0,3889	10,33
1000	0,4036	20,66
1500	0,5709	30,99
2000	0,9794	41,32

Table 2.B: Data used for calculations of peroxide value for samples: K03, K06, K09, K14, K15, K16, S01, S02, S05, S09, S13, F03, F11, F13. Reagent blanc, Fe standard were measured at 500 nm. Slope and intercept were read from standard curve.

	Abs 500 nm	average
Reagent blank	0,1575	0,1575
Reagent blank	0,1532	
Fe standard	0,3496	0,3496
Fe standard	0,3657	
Slope		47,24
Intercept		2,2119

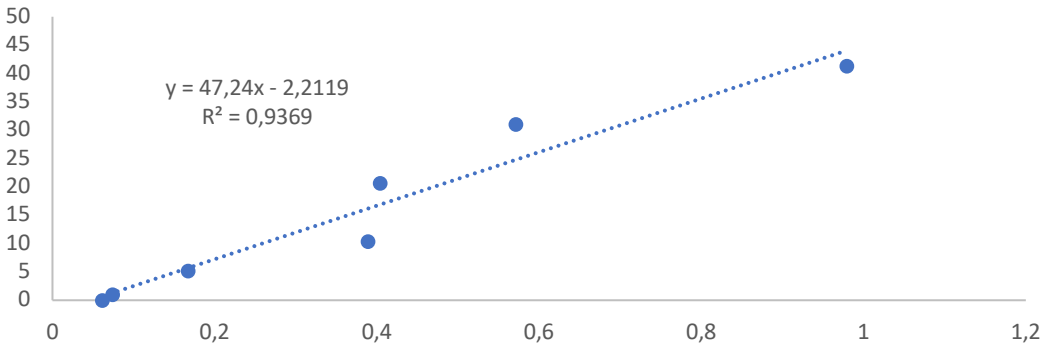

Figure 2.B: Standard curve for samples K03, K06, K09, K14, K15, K16, S01, S02, S05, S09, S13, F03, F11, F13 constructed from known concentrations of Fe(III). The value of the slope was used in order to calculate the PV.

Table 3.B: Data used for standard curve for samples: K10, K11, K18, K19S06, S07, S08, S10, S14, S15, F02, F09, F10, where measurements were based on known concentrations of Fe(III). Obtained value of the slope was used for the calculations of peroxide value.

ul stock solution	Abs 500 nm	(ul Fe(III)/10ml)^3
0	0,0472	0
50	0,0789	1,03
250	0,2394	5,16
500	0,3842	10,33
1000	0,725	20,66
1500	0,7752	30,99
2000	1,0166	41,32

Table 4.B: Data used for calculations of peroxide value for samples: K10, K11, K18, K19S06, S07, S08, S10, S14, S15, F02, F09, F10. Reagent blanc, Fe standard were measured at 500 nm. Slope and intercept were read from standard curve.

	Abs 500 nm	average
Reagent blanc	0,1844	0,20005
Reagent blanc	0,2157	
Fe standard	0,4392	0,8446
Fe standard	0,4054	
Slope		41,455
Intercept		3,7033

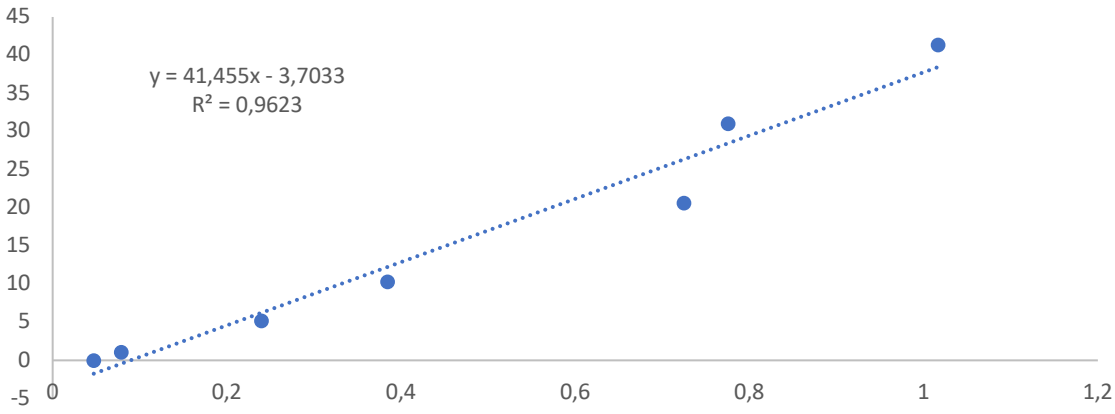

Figure 3.B: Standard curve for samples K10, K11, K18, K19S06, S07, S08, S10, S14, S15, F02, F09, F10 constructed from known concentrations of Fe(III). The value of the slope was used in order to calculate the PV.

Table 5.B: Data used for standard curve for samples: K07, K17, S07, S08 S15, S02, S09, where measurements were based on known concentrations of Fe(III). Obtained value of the slope was used for the calculations of peroxide value.

ul stock solution	Abs 500 nm	(ul Fe(III)/10ml)^3
0	0,0463	0
50	0,0979	1,03
250	0,2733	5,16
500	0,4727	10,33
1000	0,8277	20,66
1500	1,1365	30,99
2000	1,3015	41,32

Table 6.B: Data used for calculations of peroxide value for samples: K07, K17, S07, S08 S15, S02, S09. Reagent blanc, Fe standard were measured at 500 nm. Slope and intercept were read from standard curve.

	Abs 500 nm	average
Reagent blank	0,1983	0,19833
Fe standard	0,3236	0,32356
Slope		31,383
Intercept		2,9909

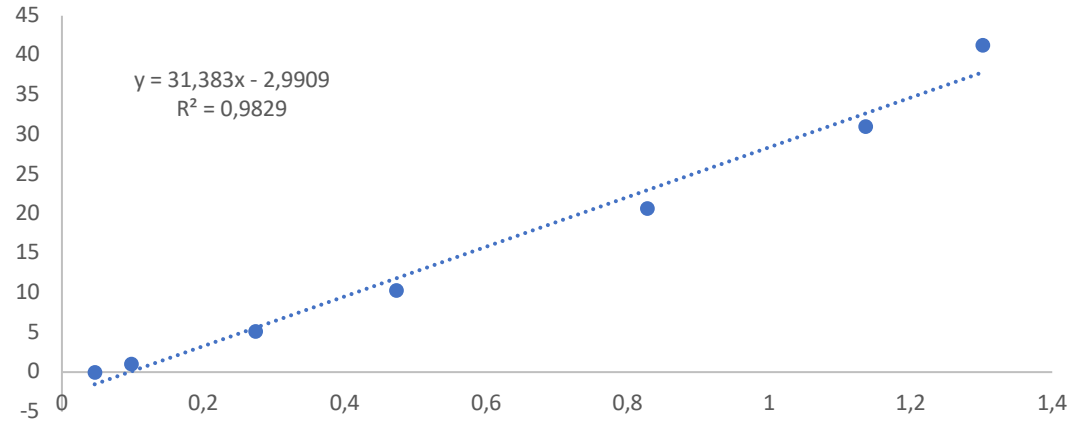

Figure 4.B: Standard curve for samples K07, K17, S07, S08 S15, S02, S09 constructed from known concentrations of Fe(III). The value of the slope was used in order to calculate the PV.

Table 7. B: Data from determination of peroxide value given in meqO2 / kg oil and standard deviation in chilled mackerel stored for 0, 2, 5 and 7 days.

day	sample	Abs 500 nm	m (g/1ml lipid)	Dilution factor	Pv [meqO2 / kg oil]	Average sample	Total average	Standard deviation
0 (25.01. 2017)	K031	0,7018	0,05475		4,204466792	3,813849411	3,042562498	0,678051049
	K032	0,6124	0,0562		3,42323203			
	K141	0,5273	0,05975		2,61749411	2,773497763		
	K142	0,579	0,06085		2,929501416			
	K161	0,5392	0,0592		2,726824385	2,540340321		
	K162	0,4792	0,0578		2,353856257			
2 (27.01. 2017)	K071	0,480923	0,0601	2	2,642154487	3,950046595	4,318983093	0,892125571
	K072	0,51507	0,03385	2	5,257938702			
	K101	0,4643	0,0621	2	3,158472775	3,670503307		
	K102	0,5311	0,05875	2	4,182533839			
	K111	0,5752	0,05585	2	4,985807601	5,336399377		
	K112	0,6429	0,0578	2	5,686991153			
5 (30.01. 2017)	K061	0,648	0,05015		4,136421532	4,403595124	4,016489069	0,581465848
	K062	0,6705	0,04645		4,670768715			
	K091	0,6397	0,05905		3,453536258	3,347842146		
	K092	0,6328	0,062		3,242148035			
	K151	0,7346	0,0598		4,081375998	4,298029937		
	K152	0,7505	0,05555		4,514683877			
7 (01.02. 2017)	K171	0,550725	0,05905	2	3,353367224	4,384799401	5,821238741	1,246528203
	K172	0,784855	0,06085		5,416231579			
	K181	0,6784	0,04855	2	7,313247748	6,61891958		
	K182	0,6183	0,0524	2	5,924591412			
	K191	0,6499	0,05545	2	6,021711108	6,459997242		
	K192	0,7507	0,05925	2	6,898283377			

Table 8. B: Data from determination of peroxide value given in meqO2 / kg oil and standard deviation in superchilled mackerel stored for 2, 5, 9 and 14 days. The red value is not taken for further calculations

day	sample	Abs 500 nm	m (g/1ml lipid)	Dilution factor	Pv [meqO2 / kg oil]	Average sample	Total average	Standard deviation
2 (27.01. 2017)	S011	0,6569	0,0615		3,434236013	3,282414125	4,568953991	1,224730685
	S012	0,6294	0,06375		3,130592238			
	S041	0,9246	0,05535		5,861261169	5,720728231		
	S042	0,9241	0,0581		5,580195292			
	S051	0,7706	0,05315		4,878482631	4,703719616		
	S052	0,7588	0,05615		4,528956601			
5 (30.01. 2017)	S071	0,744525	0,0436	2	7,039349209	7,365411603	6,434149614	0,815145735
	S072	0,81155	0,0448	2	7,691473998			
	S081	0,8313	0,0525	2	6,774778534	5,850087629		
	S082	0,680425	0,055	2	4,925396723			
	S151	0,800075	0,05555	2	6,086949609	6,086949609		
	S152	0,500475	0,05235	2	3,243172174			
9 (03.02. 2017)	S061	0,7391	0,04885	2	8,190647287	7,856556284	5,807855158	1,776803588
	S062	0,7083	0,05015	2	7,522465281			
	S101	0,5653	0,05795	2	4,678326483	4,687855432		
	S102	0,5728	0,0589	2	4,697384381			
	S141	0,5783	0,0609	2	4,610153502	4,879153757		
	S142	0,5999	0,05765	2	5,148154012			
14 (08.02. 2017)	S021	0,602525	0,05455	2	4,16358525	4,635137259	3,536444887	1,048704366
	S022	0,724525	0,0579	2	5,106689268			
	S091	0,37959	0,0446	2	2,283696553	2,546148418		
	S092	0,413505	0,04305	2	2,808600283			
	S131	0,6456	0,052	2	3,969741065	3,428048986		
	S132	0,50625	0,0511	2	2,886356907			

Table 9. B: Data from determination of peroxide value given in meqO2 / kg oil and standard deviation in frozen mackerel stored for 2 days, 8 months and one year.

day	sample	Abs 500 nm	m (g/1ml lipid)	Dilution factor	Pv [meqO2 / kg oil]	Average sample	Total average	Standard deviation
2 (27.01. 2017)	F031	0,4674	0,052		2,07799394	1,795439879	1,558122968	0,332258176
	F032	0,4006	0,05275		1,512885819			
	F111	0,468	0,04955		2,185861473	1,700531569		
	F112	0,3662	0,0537		1,215201666			
	F131	0,4017	0,0567		1,415695544	1,178397455		
	F132	0,3344	0,05505		0,941099367			
8 months (27.09. 2017)	F021	0,6654	0,0791	2	4,366736405	3,675769915	4,173453063	0,437785551
	F022	0,4365	0,0588	2	2,984803425			
	F091	0,5549	0,05915	2	4,452908822	4,345549596		
	F092	0,5238	0,0567	2	4,238190369			
	F101	0,5374	0,054	2	4,637038446	4,499039679		
	F102	0,4853	0,04855	2	4,361040912			
1 year (19.01. 2018)	F011	0,387433	0,0489	2	2,173005567	2,997063731	2,476986389	0,559701977
	F012	0,523038	0,04775	2	3,821121896			
	F061	0,402758	0,0486	2	2,363607666	2,235832247		

	F062	0,392848	0,05185	2	2,108056828			
	F121	0,48397	0,05075	2	3,162672754	2,8724379		
	F122	0,440735	0,05275	2	2,582203046			
	F161	0,363913	0,0751	2	1,238930913	1,802611678		
	F162	0,419203	0,05245	2	2,366292443			

8.3. Appendix C. TBARS in methanol/water phase

Appendix C contains experimental data from determination of TBARS in methanol/water phase in chilled mackerel stored for 0, 2, 5 and 7 days; superchilled mackerel stored for 2, 5, 9 and 14 days; and frozen mackerel stored for 2 days, 8 months and one year. The results are calculated by using the equation 2.3. The raw data and calculated TBARS are presented in Table 9.B, 10.B and 11.B. TBARS in methanol/water phase is expressed as (nmol TBARS/g muscle). Standard curves used for calculations are presented in Figure 4. C and 5 C, whereas data used for standard curve are presented in Tables 3.B – 8.B.

Table 1. C: Data used for calculations of standard curve for TBARS in methanol/water phase for samples: K03, K06, K09, K14, K15, K16, S01, S02, S04, S05, S09, S13, F03, F11, F13.

ul TEP solution	nmol TEP	Abs 532 nm
0	0	0,0624
25	2,5	0,0999
50	5	0,1452
100	10	0,3378
150	15	0,3452
200	20	0,62

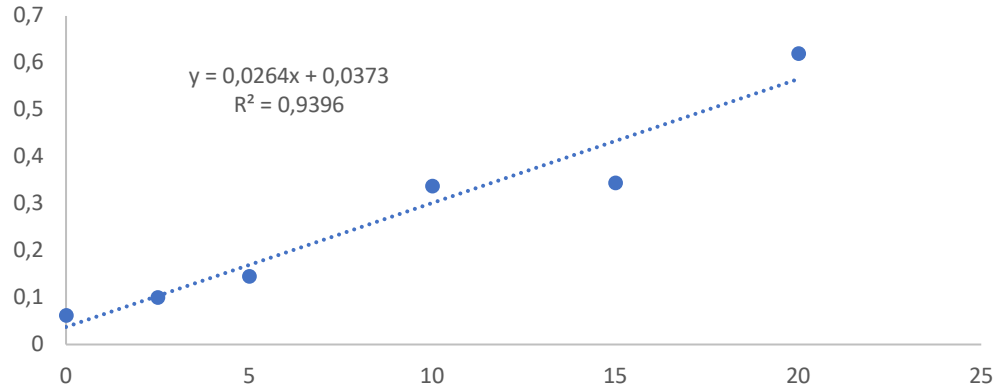

Figure 1. C: Standard curve for samples K03, K06, K09, K14, K15, K16, S01, S02, S04, S05, S09, S13, F03, F11, F13, constructed from known concentrations of TEP. The values of the slope and intercept was used in order to calculate the TBARS in methanol/water phase.

Table 2.C: Data used for calculations of standard curve for TBARS in methanol/water phase for samples: K07, K10, K11, K17, K18, K19, S06, S07, S08, S10, S14, S15, F02, F09, F10, F01, F06, F12, F16.

ul TEP solution	nmol TEP	Abs 532 nm
0	0	0,126523
25	2,5	0,147963
50	5	0,20812
100	10	0,43406
150	15	0,4789
200	20	0,66065

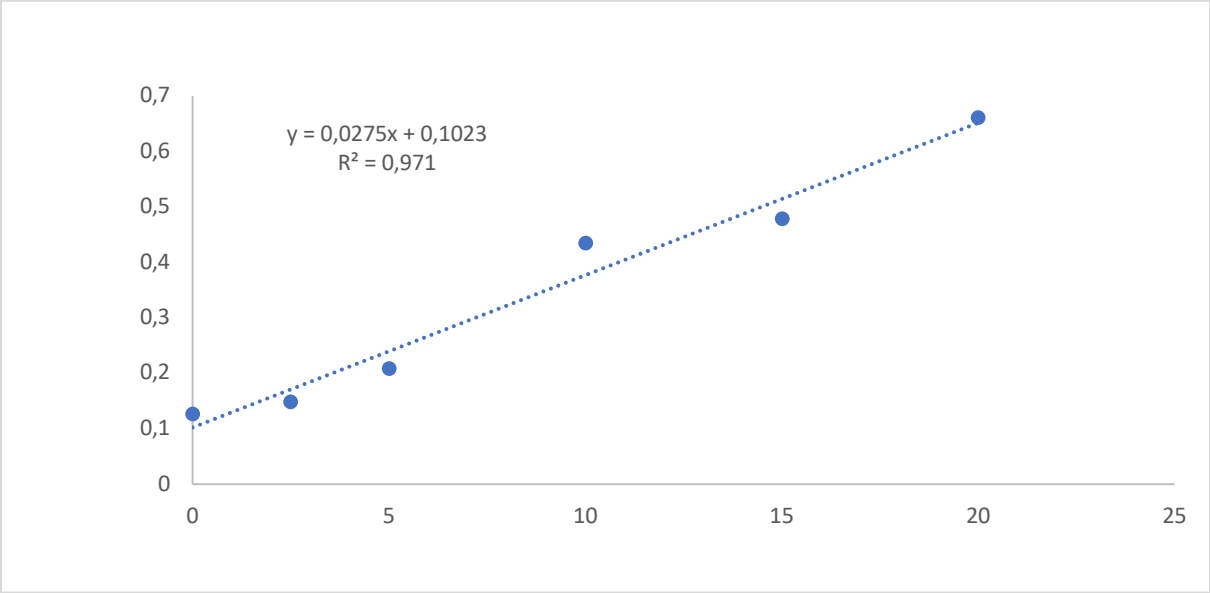

Figure 2. C: Standard curve for samples K07, K10, K11, K17, K18, K19, S06, S07, S08, S10, S14, S15, F02, F09, F10, F01, F06, F12, F16, constructed from known concentrations of TEP. The values of the slope and intercept was used in order to calculate the TBARS in methanol/water phase.

Table 3.C: Data from determination of TBARS in methanol/water phase given in nmol TBARS/g muscle and standard deviation in chilled mackerel stored for 0, 2, 5 and 7 days. The red value is not taken for further calculations.

day	sample	Abs 532 nm	Fish weight (g)	water for balance (ml)	TBARS met [nmol TBARS / g muscle]	Average sample	Total average	Standard deviation
0 (25.01. 2017)	K031	1,2161	10,08	1	181,3368056	86,49202631	78,1569807	9,469596659
	K032	0,569	10,09		86,49202631			
	K141	0,5411	10,51		79,21460081	67,86014132		
	K142	0,3384	10,2	5	56,50568182			
	K161	0,6102	10,48		88,93202059	80,11877456		
	K162	0,4621	10,28	1,5	71,30552853			
2 (27.01. 2017)	K071	0,445635	10,08		75,11374459	75,28409067	78,0921839	16,89372427
	K072	0,443835	10,37	2,8	75,45443675			
	K101	0,67616	10,05	1	108,4421891	96,21400182		
	K102	0,51157	10,1		83,98581458			
	K111	0,48244	10,16		79,52798855			

	K112	0,23285	10,26	1,5	46,02892965	62,7784591		
5 (30.01. 2017)	K061	0,3384	10,48	1	52,28013677	39,69818293	32,3221089	6,907117568
	K062	0,146	9,73		27,11622909			
	K091	0,1338	10,32		23,86437045	26,00663551		
	K092	0,1646	10,46	1	28,14890057			
	K151	0,1776	10,06	3,5	32,16411757	31,26150814		
	K152	0,1772	10,17		30,35889872			
7 (01.02. 2017)	K171	0,21707	10,5		42,02964502	44,3779786	62,4862094	17,79655237
	K172	0,25614	10,6		46,72631218			
	K181	0,356195	10,01		63,29238035	63,12686765		
	K182	0,36597	10,48	1,5	62,96135496			
	K191	0,52794	10,09	0,5	86,87874583	79,95378183		
	K192	0,43254	10,12		73,02881782			

Table 4.C: Data from determination of TBARS in methanol/water phase given in nmol TBARS/g muscle and standard deviation in superchilled mackerel stored for 2, 5, 9 and 14 days. The red values are not taken for further calculations.

day	sample	Abs 532 nm	Fish weight (g)	water for balance (ml)	TBARS met [nmol TBARS / g muscle]	Average sample	Total average	Standard deviation
2 (27.01. 2017)	S011	0,7359	10,56	0,8	106,5013774	88,36843241	86,46193834	2,696189761
	S012	0,4892	10,79		70,2354874			
	S041	0,8291	10,1	0,5	124,2866787	124,2866787		
	S042	1,0085	10,18	4	155,6527951			
	S051	0,5883	10,1		89,15691569	84,55544428		
	S052	0,5285	10,32	1	79,95397287			
5 (30.01. 2017)	S071	0,798475	10,1	1	124,859901	126,1242632	111,7836596	13,84335535
	S072	0,84817	10,31		127,3886253			
	S081	0,624755	10,15		98,98106583	110,7288142		
	S082	0,759935	10,24	4	122,4765625			
	S151	0,62638	10,44	0,5	97,08119122	98,49790161		
	S152	0,62872	10,11		99,914612			
9 (03.02. 2017)	S061	0,6969	10,2	2	111,1187166	99,5938594	96,54604096	9,257508838
	S062	0,550975	10,25		88,06900222			
	S101	0,798145	10,14		122,7072261	103,8953821		
	S102	0,5088	9,99	0,5	85,08353808			
	S141	0,54077	10,27		86,52442241	86,14888138		
	S142	0,5098	10,38	4	85,77334034			
14 (08.02. 2017)	S021	0,6795	10,02		102,9698179	102,6280156	111,7299025	12,87201192
	S022	0,6913	10,28	0,2	102,2862133			
	S091	0,1702	10,5	0,5	28,63230519	27,73434898		
	S092	0,1566	10,4		26,83639277			
	S131	0,8107	10,18		119,9023635	120,8317895		
	S132	0,7944	10,22	3	121,7612154			

Table 5.C: Data from determination of TBARS in methanol/water phase given in nmol TBARS/g muscle and standard deviation in frozen mackerel stored for 2 days, 8 months and one year. The red value is not taken for further calculations.

day	sample	Abs 532 nm	Fish weight (g)	water for balance (ml)	TBARS met [nmol TBARS / g muscle]	Average sample	Total average	Standard deviation
2 (27.01. 2017)	F031	0,283	10,82		42,60978547	38,17842611	41,23124875	2,645746737
	F032	0,1928	10,46	5	33,74706675			
	F111	0,2681	10,05		43,74038896	42,65675784		
	F112	0,268	10,64	0,5	41,57312671			
	F131	0,2396	10,33		38,58356068	42,85856229		
	F132	0,2994	10,35	0,5	47,1335639			
8 months (27.09. 2017)	F021	0,40335	10,43	5	71,39832651	74,43984122	78,6990942	14,81234162
	F022	0,493185	10,62		77,48135593			
	F091	0,37147	10,69	1,3	62,28832554	66,48300574		
	F092	0,4322	10,45		70,67768595			
	F101	0,67616	10,32	2,8	108,073728	95,17443563		
	F102	0,51157	10,31		82,27514329			
1 year (19.01. 2018)	F011	0,62505	10,12	1	100,6215415	97,53204005	89,35188449	15,4322053
	F012	0,58595	10,07		94,44253859			
	F061	0,67386	10,24	0,1	104,8753125	98,97190342		
	F062	0,57909	10,21	0,7	93,06849435			
	F121	0,434345	10,46		70,89348166	71,55171001		
	F122	0,411695	10,03	1,5	72,20993837			
	F161	0,2599	10,51	2	48,87397284	50,64630909		
	F162	0,28059	10,12	0,2	52,41864535			

8.4. Appendix D. TBARS in chloroform phase

Appendix D contains experimental data from determination of TBARS in chloroform phase in chilled mackerel stored for 0, 2, 5 and 7 days; superchilled mackerel stored for 2, 5, 9 and 14 days; and frozen mackerel stored for 2 days, 8 months and one year. The results are calculated by using the equation 2.2. The raw data and calculated TBARS are presented in Table 9.B, 10.B and 11.B. TBARS in chloroform phase is expressed as (µmol TBARS/g lipid). Standard curves used for calculations are presented in Figure 1.B, 2.B and 3.B, whereas data used for standard curve are presented in Tables 3.B – 8.B.

Table 1.D: Data used for calculations of standard curve for TBARS in chloroform phase for samples: K03, K06, K07, K09, K10, K11, K14, K15, K16, K17, K18, K19, S01, S04, S05, S07, S08, S15, S06, S10, S14, S02, S09, S13, F03, F11, F13.

ul TEP solution	nmol TEP	Abs 532 nm
0	0	0,0581
25	2,5	0,0896
50	5	0,1231
100	10	0,1679
150	15	0,2364
200	20	0,3017

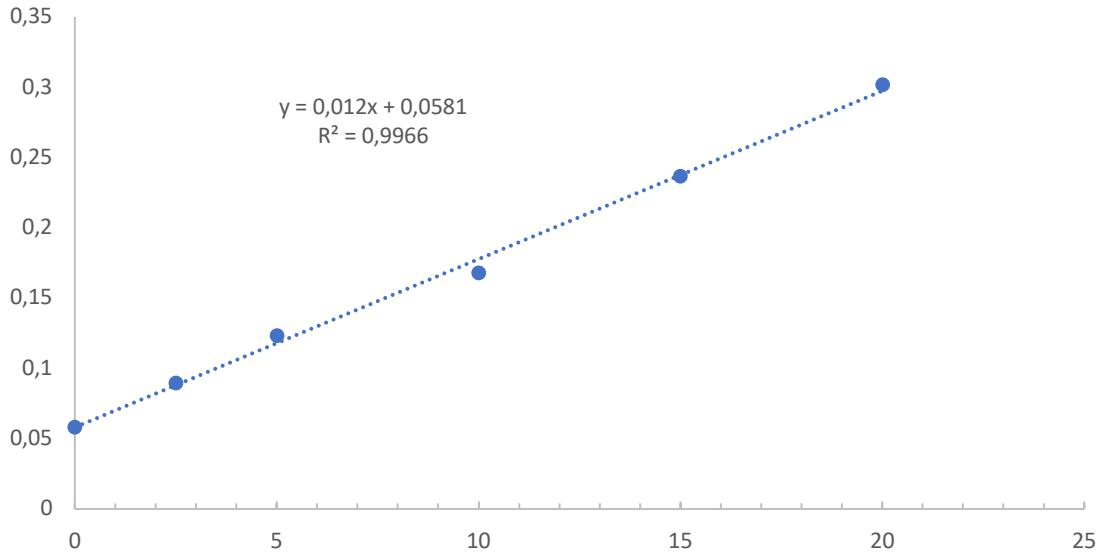

Figure 1.D: Standard curve for samples K03, K06, K07, K09, K10, K11, K14, K15, K16, K17, K18, K19, S01, S04, S05, S07, S08, S15, S06, S10, S14, S02, S09, S13, F03, F11, F13 constructed from known concentrations of TEP. The values of the slope and intercept was used in order to calculate the TBARS in chloroform phase.

Table 2.D: Data used for calculations of standard curve for TBARS in chloroform phase for samples: F02, F09, F10.

ul TEP solution	nmol TEP	Abs 532 nm
0	0	0,0602
25	2,5	0,089
50	5	0,1146
100	10	0,1704
150	15	0,1603
200	20	0,2783

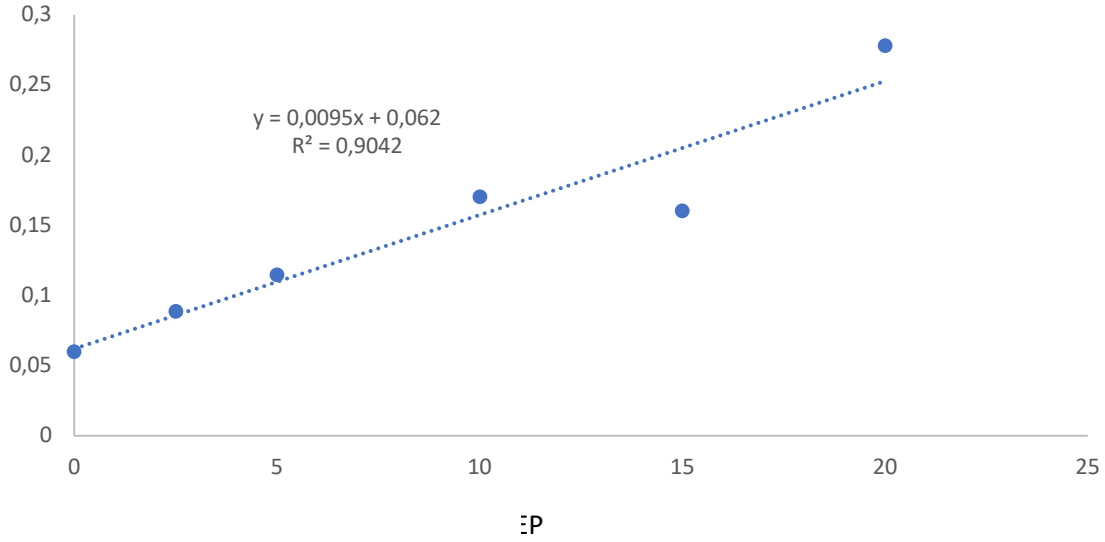

Figure 2.D: Standard curve for samples F02, F09, F10 constructed from known concentrations of TEP. The values of the slope and intercept was used in order to calculate the TBARS in chloroform phase.

Table 3.D: Data used for calculations of standard curve for TBARS in chloroform phase for samples: F01, F06, F12, F16.

ul TEP solution	nmol TEP	Abs 532 nm
0	0	0,07261
25	2,5	0,15294
50	5	0,1595
100	10	0,2191
150	15	0,4572
200	20	0,63708

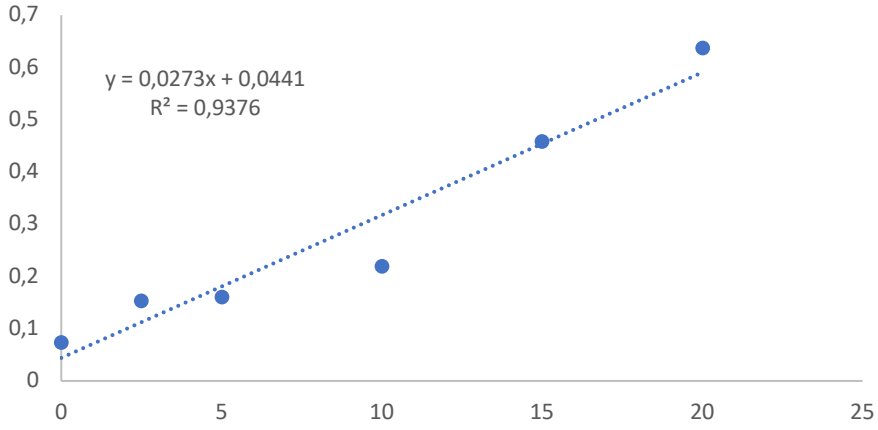

Figure 3.D: Standard curve for samples F01, F06, F12, F16 constructed from known concentrations of TEP. The values of the slope and intercept was used in order to calculate the TBARS in chloroform phase.

Table 4.D: Data from determination of TBARS in chloroform phase given in umol TBARS/ g lipid and standard deviation in chilled mackerel stored for 0, 2, 5 and 7 days. The red value is not taken for further calculations.

day	sample	Abs 538 nm	m (g/200ul lipid)	TBARS chlor. [umol TBARS/ g lipid]	Average sample	Total average	Standard deviation
0 (25.01. 2017)	K031	0,151	0,01095	0,707001522	0,665630061	0,514965758	0,057937063
	K032	0,1423	0,01124	0,6242586			
	K141	0,1453	0,01195	0,608089261	0,473998068		
	K142	0,10774	0,01217	0,339906875			
	K161	0,1326	0,01184	0,524352477	0,555933448		
	K162	0,1396	0,01156	0,587514418			
2 (27.01. 2017)	K071	0,3372	0,01202	1,934969495	1,294928607	0,59405619	0,09563201
	K072	0,1633	0,00677	1,294928607			
	K101	0,1609	0,01242	0,689747719	0,526434143		
	K102	0,1093	0,01175	0,363120567			
	K111	0,1462	0,01117	0,657266488	0,661678227		
	K112	0,1505	0,01156	0,666089965			
5 (30.01. 2017)	K061	0,3384	0,01003	2,328846793	0,788482239	0,758803006	0,121768177
	K062	0,146	0,00929	0,788482239			
	K091	0,1338	0,01181	0,534151849	0,624938828		
	K092	0,1646	0,0124	0,715725806			
	K151	0,1776	0,01196	0,832636566	0,86298795		
	K152	0,1772	0,01111	0,893339334			
7 (01.02. 2017)	K171	0,1222	0,01181	0,45230031	0,548322163	1,13064347	0,212401317
	K172	0,1522	0,01217	0,644344015			
	K181	0,1242	0,00971	0,567284586	0,740464812		
	K182	0,173	0,01048	0,913645038			
	K191	0,1935	0,01109	1,017433123	0,972499965		
	K192	0,43254	0,01185	0,927566807			

Table 5.D: Data from determination of TBARS in chloroform phase given in umol TBARS/ g lipid and standard deviation in superchilled mackerel stored for 2, 5, 9 and 14 days. The red value is not taken for further calculations.

day	sample	Abs 538 nm	m (g/200ul lipid)	TBARS chlor. [umol TBARS/ g lipid]	Average sample	Total average	Standard deviation
2 (27.01. 2017)	S011	0,1654	0,0123	0,72696477	0,691913757	1,115970104	0,517257612
	S012	0,1586	0,01275	0,656862745			
	S041	0,2829	0,01107	1,692261367	1,692261367		
	S042	0,3632	0,01162	2,188037866			
	S051	0,1723	0,01063	0,895264973	0,963735188		
	S052	0,1972	0,01123	1,032205402			
5 (30.01. 2017)	S071	0,2751	0,00872	2,073776758	2,603108617	2,353810616	0,455328476
	S072	0,3949	0,00896	3,132440476			
	S081	0,4011	0,0105	2,722222222	2,630050505		
	S082	0,3931	0,011	2,537878788			
	S151	0,2437	0,01111	1,392139214	1,828272727		
	S152	0,3426	0,01047	2,26440624			

9 (03.02. 2017)	S061	0,1837	0,00977	1,071306721	1,332014278	1,032437636	0,308127007
	S062	0,2498	0,01003	1,592721834			
	S101	0,213	0,01159	1,113747483	1,048879967		
	S102	0,1972	0,01178	0,98401245			
	S141	0,1584	0,01218	0,686234264	0,716418664		
	S142	0,1614	0,01153	0,746603064			
14 (08.02. 2017)	S021	0,1654	0,01091	0,819584479	0,914973587	1,149438731	0,331583786
	S022	0,1985	0,01158	1,010362694			
	S091	0,0842	0,00892	0,243834081	0,203701787		
	S092	0,075	0,00861	0,163569493			
	S131	0,21445	0,0104	1,252804487	1,383903874		
	S132	0,2439	0,01022	1,515003262			

Table 6.D: Data from determination of TBARS in chloroform phase given in umol TBARS/ g lipid and standard deviation in frozen mackerel stored for 2 days, 8 months and one year. The red value is not taken for further calculations.

day	sample	Abs 538 nm	m (g/200ul lipid)	TBARS chlor. [umol TBARS/ g lipid]	Average sample	Total average	Standard deviation
2 (27.01. 2017)	F031	0,1184	0,0104	0,483173077	0,295299019	0,230705766	0,069698377
	F032	0,0717	0,01055	0,107424961			
	F111	0,0791	0,00991	0,176589304	0,239986148		
	F112	0,0972	0,01074	0,303382992			
	F131	0,07936	0,01134	0,156231628	0,156832133		
	F132	0,0789	0,01101	0,157432637			
8 months (27.09. 2017)	F021	0,1728	0,01582	0,737241333	0,758434487	0,695746975	0,131249216
	F022	0,1491	0,01176	0,779627641			
	F091	0,1198	0,01183	0,51430351	0,544908183		
	F092	0,124	0,01134	0,575512856			
	F101	0,1361	0,0108	0,722222222	0,783898254		
	F102	0,14	0,00971	0,845574286			
1 year (19.01. 2018)	F011	0,11169	0,00978	0,253151756	0,188588037	0,148506397	0,066640633
	F012	0,076435	0,00955	0,124024318			
	F061	0,100965	0,00972	0,21429702	0,185352049		
	F062	0,088379	0,01037	0,156407077			
	F121	0,32493	0,01015	1,013479132	0,767923879		
	F122	0,19455	0,01055	0,522368627			
	F161	0,065366	0,01502	0,051862474	0,071579107		
	F162	0,070245	0,01049	0,09129574			

8.5. Appendix E. Color measurements

Appendix E contains experimental data from color measurements in chilled mackerel stored for 0, 2, 5 and 7 days and superchilled mackerel stored for 2, 5, 9 and 14 days. The results are used for calculation total color difference by using the equation 2.6.

*Table 1.E: Color measurements for chilled and superchilled samples, where ** C- chilled, S- superchilled and *** mean value ± standard error of mean (SEM).*

No sample	Storage regime/days**	Lightness (L*)	Redness (a*)	Yellowness (b*)	Total color difference (ΔE)
1	C, S/0	41.42±0.65***	4.43±0.25	3.28±0.13	-
2	C/2	49.11±0.93	3.86±0.38	3.96±0.19	7.74±0.15
3	C/5	53.02±0.85	3.66±0.34	5.43±0.19	11.82±0.15
4	C/7	53.06±0.83	3.47±0.31	5.48±0.23	11.88±0.17
5	S/2	54.46±0.79	3.31±0.25	5.67±0.19	13.31±0.18
6	S/5	54.45±0.96	3.22±0.27	5.73±0.30	13.31±0.21
7	S/9	54.45±0.81	2.34±0.17	5.57±0.19	13.20±0.26
8	S/14	53.45±0.85	2.20±0.15	5.45±0.27	12.42±0.28