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The Stabilizing Effect of Antioxidants in Fish Oils

Master's thesis in Biotechnology

Supervisor: Turid Rustad

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NTNU
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Summary

A dietary intake of essential long chain omega-3 polyunsaturated fatty acids (LC omega 3PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are important in human nutrition to maintain good health. Research have shown that elevated intake of LC omega-3 PUFAs can give health promoting effects, and the dietary recommendations given by EFSA is 250-500 mg/day (EFSA, 2012). Today the main sources of EPA and DHA for human consumption is through marine products such as fish and fish oil supplements. Unfortunately, are the products rich in PUFAs highly prone for lipid oxidation which leads to decomposition of the PUFAs and loss in nutritional value. To limit the lipid oxidation of PUFAs, factors such as temperature, light, oxygen, fatty acid composition and the presence of pro- and antioxidants must be evaluated and controlled. To be able to do this more knowledge about the oxidation reactions and the use of antioxidants for prevention is needed. Considering this, the aim of this thesis has been to study the lipid oxidation reactions and the stabilising effects of different antioxidants in different types of fish oils.

The accelerated oxidation test, Schaal oven test, was used to study the oxidative stability of refined cod liver oil at different storage temperatures. The different storage temperatures utilized in this study was 10, 22.5, 30, 40 and 50 degrees. In addition, samples periodically collected during the storage period was analysed by iodometric titration (PV) and TBARS assay to study the development of primary and secondary oxidation products, respectively. The weight increase measurements observed from the Schaal oven test showed that the weight increase rate, reflecting the lipid oxidation rate, in the samples increased along with the storage temperature. The PV and TBARS results also showed that the accumulation of oxidation products was faster at higher storage temperatures and decreased at lower temperatures.

In the antioxidant experiments the ability to provide oxidative stability was studied for six antioxidants in five different fish oils. The antioxidants utilized were rosemary extract, BHA, α-tocopherols, mixed tocopherols, A/L/T mixture and ascorbyl palmitate. The fish oils were a refined cod liver oil (TRAN), and four anchovy oils (12/18-S, 12/18-B, TG60, TG90) varying in the number of refining steps received. For each fish oil, samples containing different antioxidant additives, including a control without addition of antioxidant, were prepared. The Schaal oven test was performed at 50 degrees and the results for each sample was compared and an antioxidant potency order was established. Rosemary extract was found to perform best

in some of the fish oils, while in others the effect was found to be poor. The efficiency of the α-tocopherol additive also varied between the fish oils, but it was always found to provide a lower oxidative stability compared to the mixed tocopherol additive. The mixed tocopherol additive was in general observed to provide good oxidative stability compared to the other antioxidant additives and was, in the anchovy oils with high PUFA content (TG60, TG90), found to be the most efficient additive. The ascorbyl palmitate additive only dissolved in the cod liver oil where it provided poor, almost negligible, effect. The A/L/T mixture containing ascorbyl palmitate, lecithin and α-tocopherols was found to perform well in the initial stages of the lipid oxidation when added in the cod liver oil. However, in the anchovy oils the efficiency was reduced and it did not dissolve in the anchovy fish oil concentrates (TG60, TG90). The BHA additive showed an intermediate response compared to the other additives in all fish oil samples, but this observation may be a result of the BHA being added in a lower concentration compared to the other six additives (BHA = 150 ppm, others = 1000 ppm). An OSI test was also performed for the antioxidant samples of the cod liver oil (TRAN) and the TG60 fish oil samples, at 50 degrees. This was performed to evaluate deviations between the results obtained from the OSI and the results obtained from the Schaal oven test. However, no obvious deviations regarding the antioxidant potency order was observed between the two tests.

The observations done in this thesis, underlines the importance of always testing the antioxidant efficiency in the specific product which the antioxidant additive is attended to be added for oxidative stability reasons. This is important because, as observed in this thesis, the efficiency of an antioxidant additive in a product, does not necessarily apply for all similar products.

Sammendrag

Inntak av essensielle langkjedede omega-3 flerumettede fettsyrer, som eikosapentaensyre (EPA) og dokosaheksaensyre (DHA), gjennom kosten er viktig for å opprettholde god helse. Forskning viser at inntak av disse fettsyrene har helsefremmende effekt og EFSA anbefaler et inntak på 250-500 mg/dag (EFSA, 2012). I dag er de viktigste kildene for inntak av EPA og DHA gjennom fisk og kosttilskudd med innhold av fiskeolje. Dessverre, er produkter som inneholder store mengder flerumettede fettsyrer svært utsatt for lipid oksidasjon, som fører til tap av næringsverdi ved at fettsyrene brytes ned. For å begrense lipidoksidasjon av disse fettsyrene må faktorer som temperatur, lys, oksygen, fettsyresammensetning og tilstedeværelse av pro- og antioksidanter evalueres og kontrolleres. For å lykkes med dette er det nødvendig med mer kunnskap om reaksjonene som finner sted ved lipidoksidasjon og hvordan antioksidanter kan benyttes til å forebygge mot disse. Hensikten med denne avhandlingen har derfor vært å studere lipidoksidasjon og effekten av ulike antioksidanter når de tilsettes i ulike fiskeoljer.

En akselerert oksidasjonstest, Schaal oven test, ble benyttet for å studere den oksidative stabiliteten til raffinert torskeleverolje ved lagring i ulike temperaturer. De ulike lagringstemperaturene benyttet i disse forsøkene var 10, 22.5, 30, 40 og 50 grader. I tillegg ble paralleller, som regelmessig ble tatt ut av eksperimentet under lagringsforsøket, analysert ved bruk av metodene jodometrisk titrering (PV) og TBARS-analyse. Dette ble gjort for å studere utviklingen av henholdsvis primære og sekundære oksidasjonsprodukter. Vektøkning som ble observert for prøvene under Schaal oven test viste at vektøkningsraten, som reflekterer lipidoksidasjonsraten, økte med lagringstemperaturen. Resultatene fra PV og TBARS viste også at akkumulering av oksidasjonsprodukter skjedde raskere i prøver lagret ved høyere temperaturer og minket med lagringstemperaturen.

I antioksidantforsøkene ble evnen til å bedre fiskeoljers oksidative stabilitet studert for seks ulike antioksidanter i fem ulike fiskeoljer. Antioksidantene som ble benyttet i forsøkene var rosmarinekstrakt, BHA, α-tokoferoler, en blanding av ulike tokoferoler, A/L/T blanding og askorbylpalmitat. Fiskeoljene var en raffinert torskelever olje (TRAN) og fire ansjosoljer (12/18-S, 12/18-B, TG60, TG90). De fire ansjosoljene varierte i antall behandlings steg de var eksponert for. For hver fiskeolje ble det forberedt prøver med tilsetning av ulike antioksidanter og en kontroll uten tilsatt antioksidant. Schaal oven test ble utført ved lagring i 50 grader og

resultatene for hver prøve ble sammenlignet for å rangere i hvilken grad de ulike antioksidantene hadde hatt effekt i forhold til hverandre. Rosemarinekstrakt var observert å fungere best i noen fiskeoljer, mens i andre ga ekstraktet dårlig effekt. Effekten av α-tokoferolene varierte også mellom de ulike fiskeoljene, men ble alltid funnet å ha lavere effekt sammenlignet med blandingen av ulike tokoferoler. Blandingen med ulike tokoferoler ble observert å generelt gi god effekt sammenlignet med de andre antioksidantene og ble funnet å være den mest effektive antioksidanten i ansjosolje med høyt innhold av flerumettede fettsyrer (TG60, TG90). Askorbylpalmitat løste seg kun i torskeleveroljen (TRAN) og ble her observert med dårlig effekt. A/L/T blandingen som inneholder askorbylpamitat, lecitin og α-tokoferol ble observert å ha god effekt i tidlige faser av lipidoksidasjonen når tilsatt i torskeleveroljen. I ansjosoljene var effekten redusert og i fiskeoljekonsentratene (TG60, TG90) ville ikke blandingen løse seg. BHA ble observert med en generell middels god effekt sammenlignet med de andre antioksidantene i alle fiskeoljene, men dette resultatet kan komme av at BHA under eksperimentet ble tilsatt til fiskeoljene i en lavere konsentrasjon (BHA = 150 ppm, andre = 1000 ppm). En OSI-test ble også utført for antioksidant prøvene av fiskeoljene TRAN og TG60. OSI testen ble som Schaal oven testen også utført ved 50 grader. OSI-testen ble gjennomført for å evaluere for eventuelle avvik mellom resultatene observert fra Schaal oven test og OSI, men ingen åpenbare forskjeller i antioksidant effekt ble observert.

Observasjonene gjort i denne avhandlingen understreker viktigheten av å alltid teste effekten av antioksidanter i det spesifikke produktet hvor de skal tilsettes og er tiltenkt å øke den oksidative stabiliteten. Dette er viktig, for som observert i denne avhandlingen, vil ikke effekten observert i et produkt nødvendigvis være den samme i lignende produkter.

Preface

This master thesis was written as part of the MSc Biotechnology at Norwegian University of Science and Technology, NTNU. The work was performed at the Department of Biotechnology, NTNU, and at SINTEF Ocean both located in Trondheim, Norway.

First, I would like to thank my supervisor Professor Turid Rustad at the Department of Biotechnology, NTNU for her always admirable quick e-mail responses, for guidance in scientific writing and for taking the time to proofread my thesis. Thank you also for being a committed supervisor for your master students, and in that way making our last year a lot easier.

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I would also express my gratitude to all the amazing people I have met during my five years in Trondheim. It has been a blast! Finally, thank you to Glenn and my family for always supporting me and cheering me on.

Mina Oline Odden

Trondheim 1th of July 2020

Abbreviations

ALA – Alpha-linolenic acid

AOCS – American Oil Chemists Society

A/L/T – Ascorbyl palmitate, Lecithin and Tocopherol

AP – Ascorbyl palmitate

AV – Anisidine value

BHA - Butylated hydroxyanisole

BHT – Butylated hydroxytulene

DHA – Docosahexaenoic acid

EDTA – Ethylenediaminetetraacetic acid

EE – Ethyl ester

EFSA – European Food Safety Authority

EPA – Eicosapentaenoic acid

FA - Fatty acid

FID – Flame ionisation detector

GC – Gas chromatography

HPLC – High pressure liquid chromatography

KI – Potassium iodine

LA - Linoleic acid

MA – Malonaldehyde

No AOX – Without antioxidant

OSI – Oxidative stability index

PCB – Polychlorinated biphenyl

PUFA – Polyunsaturated fatty acid

PV - Peroxide value

R&D – Research and Development

SD – Standard deviation

TBA - Thiobarbituric acid

TBARS – Thiobarbituric acid reactive substances

TEP – 1,1,3,3-tetraethoxypropane

TG - Triglyceride

Content

1.Introduction

1.1 General background

Fish oils only make up about 10% of the edible oil production in the world (Mozuraityte, Kristinova, Standal, Carvajal, & Aursand, 2016). Still, fish oils are of high importance in human nutrition because of the high content of essential polyunsaturated fatty acids (PUFAs). Unfortunately, the utilization of PUFAs is limited by the lipid oxidation reactions which leads to degradation of the valuable PUFAs (Frankel, 2005).

A PUFA is a fatty acid containing multiple double bonds along the hydrocarbon chain. The PUFAs are usually categorized into omega-3- and omega-6 fatty acids. In the omega-6 fatty acids the first double bond is located at the sixth carbon counting from the methyl end. In the omega-3 fatty acids the first double bond is located at the third carbon. The three most important omega-3 fatty acids for human health are the alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALAs is supplied through dietary sources such as walnuts, chia and green leafy vegetables and are the precursor of the endogenous synthesis of EPA and DHA (Kamal-Eldin & Yanishlieva, 2002; Simopoulos, 2016). Unfortunately, this synthesis is slow and the EPA and DHA are also considered essential fatty acids which should be supplied through the diet to ensure healthy levels. The slow synthesis of EPA and DHA from ALA is caused by the catalysing enzymes of the synthesis having higher affinity for linoleic acid (LA), which is the precursor of the pathway synthesizing omega-6 fatty acids. The omega-6 fatty acids are like the omega-3 fatty acid synthesised through desaturation and elongation reactions. The higher affinity for LA, combined with a higher dietary intake of LA compared to ALA for most people, results in a slow endogenous anabolism of EPA and DHA (Simopoulos, 2016). For that reason, the main sources of dietary EPA and DHA is today through marine products such as fish and fish oil supplements. The dietary recommendations of EPA and DHA given by EFSA is 250-500 mg/day (EFSA, 2012) and is well documented to serve health promoting effects on various diseases and risk factors. In the review published by Swanson et. al elevated intake of EPA and DHA is related to improved cardiovascular functions in terms of showing anti-inflammatory and anti-platelet effects (Swanson, Block, & Mousa, 2012). Elevated intake of EPA and DHA are also linked to improved cognitive functions in patients with Alzheimer disease and to facilitate proper fetal development in women during pregnancy. Supplement during pregnancy is also found to decrease the immune responses as

well as allergies in infants after birth (Anderson & Ma, 2009; Calder, 2013; Swanson et al., 2012; VKM, 2011a).

1.1.1 Fish oils for human consumption

About 40% of the global production of omega-3 fatty acid containing products for human consumption are made in Norway (VKM, 2011a). The crude fish oil used in the production of fish oil supplements are mostly imported from regions such as Chile and Peru, but some are also manufactured in Norway. In Norway, the crude fish oils are mostly made from cod liver and rest raw materials such as cut-offs from other fish processing lines, like salmon. However, the quality and composition of the raw materials utilized in the production of crude fish oils vary. These variations are due to factors such as storage conditions, time before processing, catch season, fishing ground, fish species and what parts of the fish are utilized, such as the whole fish, cut-offs or specific entrails like the liver. The raw materials used for crude fish oil production are composed of three fractions: solids, oil, and water, where the solids are defined as the fat-free dry matter (VKM, 2011a). Separation of the oil from the two other fractions is achieved by first cooking and/or treating the raw materials with enzymes. This step is performed to denature the fish protein to be able to liberate the oil from the fat depots in the tissue. The liquid parts of the fish are further separated from the solids by pressing. Remaining solids still present are further removed by passing the liquid over a vibrating screen with 5-6 mm perforation and then passed through a decanter for removal of fine suspended solids such as sand. To separate the oil from the water fraction a separator is used and the resulting oil fraction is further polished by repeatedly washing with hot water and separation into water and crude fish oil fractions (Breivik, 2007). Dependent on the raw material utilized and the processing conditions utilized, the overall chemical composition of the crude fish oil will vary. This includes differences in the presence of decomposition substances, oxidation products, process generated substances and fatty acid profiles (VKM, 2011a).

To make the crude fish oil suitable for human consumption it must undergo further refining steps to remove impurities. The impurities can be undesirable compounds such as PCB, waxes, free fatty acids, trace metals and oxidation products which are naturally present in the raw materials or formed during processing and storage. Steps which may be used to refine a crude fish oil includes neutralisation, degumming, winterisation, bleaching, deodorisation, stripping and distillation. After refining the fish oils usually contain about 10-30% EPA and DHA present as triglycerides. However, the yield is dependent on the raw material utilized in the production

as well as the treatments perform during refining (VKM, 2011a). Further the refined fish oils can undergo chemical modifications including steps such as transesterification and concentration by distillation. In these processing steps the existing triglycerides are broken down and re-build, resulting in an enhanced content of omega-3 fatty acids which can reach levels up to 60-90% of the total fatty acid content (VKM, 2011a).

The refined fish oil and the fish oil concentrates are utilized in food supplements and pharmaceuticals for human consumption. Unfortunately, oils containing these amounts of PUFAs are highly prone to lipid oxidation. If not controlled the lipid oxidation progresses rapidly and results in destruction of valuable PUFAs which decreases the nutritional value of the oil. Oxidized lipids can also react with other structures in the substrate such as proteins and pigments leading to texture and colour changes. Together with the off flavours produced during lipid oxidation, the palatability of the oil is reduced which consequently reduces the customer acceptance. More research is also needed regarding the effects of oxidized lipid compounds on human health, a topic of dispute and concern in the last decades (Frankel, 2005). Risk assessments regarding the consumption of oxidized fish oil was ordered by several authority organs such as the Norwegian Food Safety Authorities (VKM) and the European Food Safety Authority (EFSA)(EFSA, 2010; VKM, 2011b). However, no firm conclusions were made because the lack of published studies on this topic. For that reason, it is critical to acquire knowledge about the lipid oxidation reactions, to fully understand the parameters of influence, and thus be able to ensure high quality fish oil for human consumption.

1.2 Lipid oxidation

Lipid oxidation is the most important process which leads to degradation of fats and oils. How prone a certain fish oil is to oxidation is highly dependent on the fatty acid composition. A higher degree of unsaturation is often in accordance with lower oxidative stability (Mozuraityte et al., 2016). Other factors with effect on the susceptibility towards lipid oxidation is: the presence of pro- and antioxidants, the structural organization of the fatty acid, and the storage conditions including temperature, light and oxygen exposure (Kamal-Eldin & Yanishlieva, 2002). For oxidation of the fatty acids to occur, either the fatty acid must be converted to a radical, or the oxygen molecule must be activated. This because a direct interaction between an atmospheric oxygen molecule and an unsaturated fatty acid is impossible as a result of their orbital spin directions (Frankel, 2005; VKM, 2011a).

The fatty acids can be oxidized in different ways which are often classified according to the mechanism of action. Photooxidation is oxidation due to light exposure where oxygen is activated through photosensitization. Photosensitization is the process where a compound in the oil capable of absorbing light (a sensitizer), such as riboflavin, porphyrins and chlorophyll, absorbs light and further transfers the energy to oxygen which become activated and enters the activated (or singlet) oxygen state (Mozuraityte et al., 2016; Uluata, McClements, & Decker, 2015). The singlet oxygen is characterized by having an empty outer orbital making it highly reactive and able to interact with the unsaturated fatty acids and form fatty acid radicals. The sensitizers can also serve as prooxidants by directly interacting with the unsaturated fatty acid, causing hydrogen abstraction and thus lead to formation of a fatty acid radical (Frankel, 2005). In enzymatic oxidation the reaction is catalysed by endogenous enzymes such as lipoxygenases which incorporate molecular oxygen into the fatty acids resulting in formation of hydroperoxides (Mohd Fauzi & Spickett, 2015). However, both enzymatic oxidation and photooxidation are to a large extent avoided by protecting the substrate from direct sunlight and by inactivation of endogenous enzymes through heat treatments in the refining steps. Another mechanism of lipid oxidation is the autoxidation which is harder to avoid. The autoxidation is often referred to as spontaneous and is considered the main challenge in maintaining oxidative quality of fish oils (VKM, 2011a).

1.2.1 Autoxidation

The autoxidation is a complex chain reaction where fatty acids are activated into fatty acid radicals that can react directly with atmospheric oxygen. To get an overview of the various reactions the autooxidation is often divided into three phases which are listed below. However, the phases do overlap and the different reactions to some extent occur simultaneously (Frankel, 2005).

Initiation → Propagation → Termination

Initiation

In the initiation phase the fatty acid radicals ($R^{\cdot}$) are formed. The formation occurs in the presence of an initiator which result in abstraction of a hydrogen atom from the bis-allylic position of the fatty acid (RH) (Mohd Fauzi & Spickett, 2015).

$RH \rightarrow R^{\cdot} + H^{\cdot}$

Because of the surrounding double bonds in the PUFAs the dissociation energy for loss of hydrogen atoms at the bis-allylic carbons are low (Mozuraityte et al., 2016). According to E.N Frankel the predominant mechanism of hydrogen abstraction is resulting from hydroperoxides already present in the substrate. The hydroperoxides decompose into free radicals which further interact with the unsaturated fatty acids and result in formation of fatty acid radicals (Frankel, 2005). Other initiators of the reaction can be chemical oxidizers such as activated oxygen species ($OH^{\cdot}$, $O_2^{\cdot -}$) and transition metals mostly iron and copper. The fatty acid radicals can also be formed as a result of thermal cleavage (VKM, 2011a).

Propagation

In the propagation step the fatty acid radicals ($R^{\cdot}$) reacts with atmospheric oxygen to form peroxyl radicals ($ROO^{\cdot}$)(Frankel, 2005).

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$$

The peroxyl radical, because of its high energy, then proceeds by abstracting a hydrogen atom from another molecule. Since the energy of the carbon-hydrogen bond in the bis allylic positioned carbons of unsaturated fatty acids is low they are highly susceptible for interaction with the peroxyl radical. The hydrogen abstraction by the peroxyl radical result in formation of a hydroperoxide (ROOH), while the unsaturated fatty acid attacked results in formation of a new fatty acid radical (Damodaran, Parkin, & Fennema, 2008).

$$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$$

The newly formed fatty acid radical will start following the same pathways as already described and the propagation reactions will in this way proceed in a cyclic manner resulting in accumulation of hydroperoxides in the oxidized substrate (Frankel, 2005). This cyclic nature of the propagation reactions is illustrated in figure 1.2.1.1.

Termination

In the termination phase the accumulating radicals starts to interact with each other and forms stable non-radical products as illustrated in the reaction equations below. These termination products include small volatile compounds which give rise to the characteristic off-flavours commonly associated with rancid fish products, including fish oils (Damodaran et al., 2008). The lipid oxidation products are further described in section 1.2.3.

$R^{\cdot} + R^{\cdot} \rightarrow RR$

$ROO^{\cdot} + R^{\cdot} \rightarrow ROOR$

$ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2$

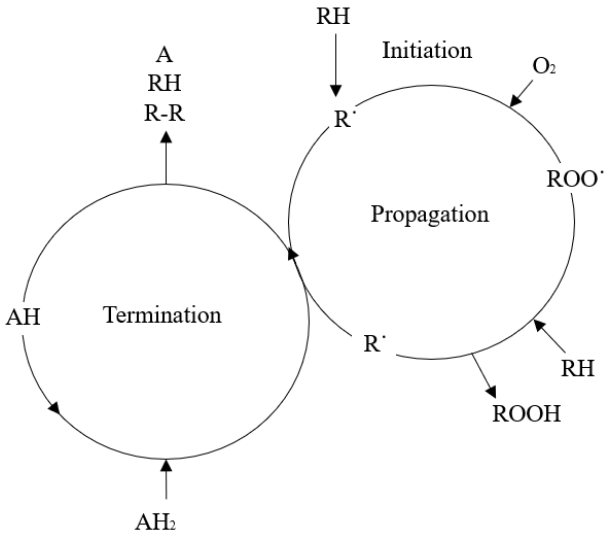

Figure 1.2.1.1: An overview of the cyclic reaction pathways of lipid oxidation. Adapted from Allen and Hamilton, 1994 (Allen & Hamilton, 1994).

1.2.2 The progression of the lipid oxidation

In the early stages of lipid oxidation almost no decomposition products are formed. The reaction rates are low, and the impacts of lipid oxidation on the substrate are almost negligible. This phase is called the induction phase (Shahidi, 2005). The length of the induction phase is highly important for food processors, because in this phase rancidity is not detectable and the quality of the products are high (Damodaran et al., 2008). Factors affecting the length of the induction phase are storage conditions, the presence of pro- and antioxidants and degree of unsaturation. Substrates with a higher degree of unsaturation are usually more prone to lipid oxidation and thus will have a shorter induction phase compared to more saturated substrates if stored at equal conditions. After the induction phase the lipid oxidation enters the exponential phase. In this phase the rate of decomposition increases sharply. The primary oxidation products accumulate first and are mainly hydroperoxides. When the hydroperoxides accumulates the nutritional quality decreases due to the loss of PUFAs. However, since most of the hydroperoxides are taste- and odourless the effects on the sensory quality is still minor (VKM, 2011a). As the lipid

oxidation proceeds the concentration of hydroperoxides eventually starts to decrease as the rate of formation falls below the rate of decomposition. The hydroperoxides are decomposed into various secondary oxidation products which will accumulate in the substrate (Frankel, 2005). The progression of lipid oxidation described in this section is illustrated in figure 1.2.2.1.

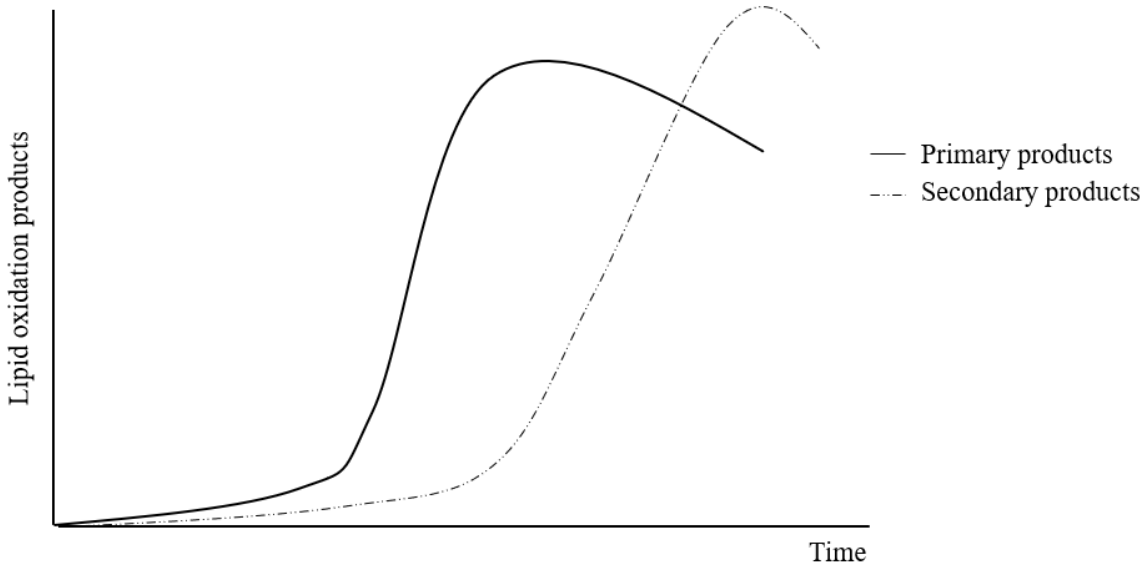

Figure 1.2.2.1: A schematic illustration of how the primary and the secondary oxidation products will accumulate in an oxidized substrate over time. Adopted from Frankel, 2005 (Frankel, 2005).

1.2.3 Lipid oxidation products

The primary oxidation products are, as described in the previous section, the lipid hydroperoxides. The hydroperoxides does not contribute to the rancid smell normally associated with oxidized fish products. However, they are not stable and will proceed to decompose into free radicals which will proceed to form secondary oxidation products. The decomposition can take place by thermal cleavage due to thermal processing of the food product or by the action of prooxidants such as transition metals. Since the decomposition leads to formation of additional free radicals the accumulation of hydroperoxides is found to most likely be responsible for the exponential increase in oxidation rate normally observed after the induction phase, as described in section 1.2.2 (Damodaran et al., 2008).

Exactly what products are formed during the decomposition of the hydroperoxides depends on several factors such as type of fatty acid, the location of the oxidation on the fatty acid and through what pathway the formed hydroperoxide is decomposed (Frankel, 2005). Several different reaction pathways can occur and the products resulting from these pathways can still

be unsaturated and for that reason also proceed to oxidize and decompose even further. In that way, the different types of secondary lipid oxidation products which can be formed are large (Damodaran et al., 2008). One type of free radicals formed during decomposition of hydroperoxides are the alkoxyl radicals (Damodaran et al., 2008). The further degradation of alkoxyl radicals can take place through several pathways, but the main pathway for decomposition of fatty acids are through the β-scission reactions, which leads to formation of many different low molecular weight compounds of which some are volatile. The volatile secondary oxidation products formed are the ones responsible for the rancid perception of oxidized fish and fish products. In the β-scission the aliphatic chain of the fatty acid is cleaved at the alkoxyl radical location. This cleavage results in an aldehyde and an alkyl radical (Figure 1.2.3.1) (Frankel, 2005).

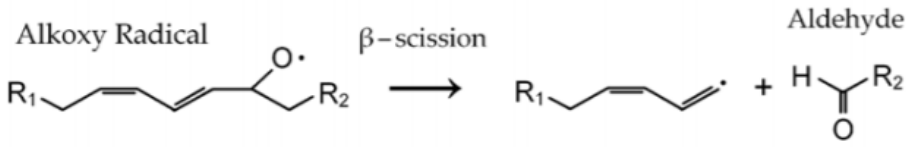

Figure 1.2.3.1: β-scission of an alkoxyl radical resulting in an alkyl radical and an aldehyde (Turner-Walker, 2012).

The aldehydes are relatively stable and can accumulate as secondary oxidation products, but they can also oxidize further to yield organic acids. The aldehydes are also found to be able to interact with the sulfhydryls and amines in protein, altering their functionality. One example is the interaction with histidine in the myoglobin which is thought to be the reactions causing discoloration in meat (Damodaran et al., 2008). The alkyl radical ($-CH_2^{\cdot}$) formed on the aliphatic chain can react with a hydrogen ($H^{\cdot}$) to form a hydrocarbon, with a hydroxyl radical ($HO^{\cdot}$) to form an alcohol or with oxygen to form a hydroperoxide. As already mentioned, the hydroperoxide can form at multiple locations along a PUFA chain. For that reason the cleavage of the fatty acid aliphatic chain through β-scission reactions can also happen at multiple locations resulting in a large number of different secondary oxidation products (Damodaran et al., 2008). An alternative pathway of which the alkoxyl radicals are decomposed into aldehydes is the Hock rearrangement. In this pathway the alkoxyl radical is cleaved resulting in two aldehyde fragments (Mohd Fauzi & Spickett, 2015). Beside the decomposition reactions leading to the formation of aldehydes, the fatty acid radicals can also proceed to react in other reaction pathways resulting in secondary oxidation products such as olefins, alcohols, carboxyl

acids, ketones, epoxides, hydroxides, ethers and cyclic products. An analysis of fish oils using dynamic headspace gas chromatography showed that the oxidized fish oil contained over 150 different volatile compounds (Frankel, 2005). Figure 1.2.3.2 provides an overview of the many different secondary oxidation products possibly formed during lipid oxidation. However, the aldehydes are often considered the most important group because of their leading role regarding the off-flavour development (Damodaran et al., 2008). Sensory detection of some of these products are prominent at concentrations as low as the ppm range (Frankel, 2005). For that reason, it is desirable to prolong the early stage of lipid oxidation, the induction phase, where the concentration of the lipid oxidation products is low, and the quality of the product such as fish oils is high. This can for instance be achieved by addition of antioxidants which stabilize the oil and reduces the oxidative damage.

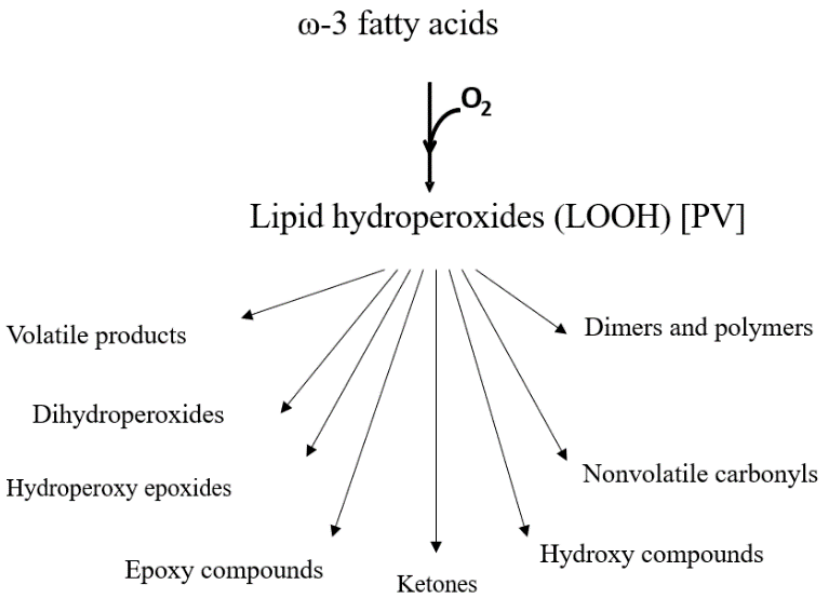

Figure 1.2.3.2: An overview of the many different secondary oxidation products potentially formed during decomposition of the hydroperoxides. Adopted from Kamal-Eldin, 2003 (Kamal-Eldin, 2003).

1.3 Antioxidants

The antioxidants are important tools used to postpone the escalation of lipid oxidation. Even though crude fish oils naturally contain antioxidants, such as tocopherols, carotenoids, amino acids and peptides, ascorbic acid, and ubiquinone, these are often removed during processing due to such as elevated temperatures, distillation, and treatment with bleaching earth. For that reason, these antioxidants do not provide sufficient protection during storage and distribution. Addition of external antioxidants to fish oils and fish oil raw materials is therefore necessary to promote the stability and extend the shelf life of the fish oils (Mozuraityte et al., 2016). By definition an antioxidant is, as quoted by Frankel and Meyer "Any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Frankel & Meyer, 2000).

The different types of antioxidants can be classified in several ways such as by source, function, mechanism of action and chemical structure. When classified by function, the different classes are (Tsao, 2015):

- Free radical scavengers
- Metal chelators
- Singlet oxygen quenchers
- Synergists / Regenerators
- Reducing agents
- Enzyme inhibitors

The free radical scavengers are antioxidants contributing by directly breaking off the chain reaction in lipid oxidation. This is achieved by the antioxidants ability to react with the free radicals before they get the change to react with the unsaturated fatty acids (Damodaran et al., 2008). These antioxidants are often referred to as the primary antioxidants (Shahidi & Zhong, 2010). The inhibition of the free radicals is through donation of a hydrogen atom or by transfer of a single electron. Both mechanisms result in free radical neutralization (Shahidi, 2015). The efficiency of a free radical scavenger is dependent on the ability to donate a hydrogen atom and increases as the bond energy of the hydrogen decreases. It is also an advantage that the antioxidant radical formed as a result of the hydrogen donation have a low energy and do not participate in the further propagation of the lipid oxidation (Damodaran et al., 2008).

Compounds shown to be effective as primary antioxidants are the phenolic compounds. Some phenolic compounds well-known for their use in food systems are tocopherols, rosemary extract and butylated hydroxyanisole (BHA). The characteristic of phenolic compounds is that they consist of one or more aromatic rings in their chemical structure. After donation of a phenolic hydrogen to neutralize a free radical, the phenolic compound become an antioxidant radical. However, through delocalization the unpaired electron on the phenolic radical is stabilized, and the reactivity is normally low preventing it from participating further in propagation of the lipid oxidation. On the other hand, the antioxidant radicals can proceed to interact with additional free radicals to form stable non-radical products. In this way, each phenolic antioxidant molecule can contribute by scavenging two free radicals (Shahidi, 2015; Shahidi & Zhong, 2010).

The other classes listed are all classified as secondary antioxidants and are characterised by having a more preventive role in prevention of the lipid oxidation. Instead of directly breaking off the chain reaction, the secondary antioxidants act by supressing initiators of oxidation. The metal chelators act by stabilizing transition metals present in the substrate, which are mostly iron and copper ions. The metal chelating activity of antioxidants can be through occupation of the metal coordination sites, formation of insoluble metal complexes or by steric prevention of interaction between the metal ions and the lipids or hydroperoxides. A metal chelation antioxidant can also work by more than one of these mechanisms and the efficiency of the metal chelator is highly dependent on the metal-to-chelator ratio within the oxidizing substrate. For that reason the addition of the antioxidants should be carefully investigated for the specific substrate at hand (Damodaran et al., 2008). Widely used metal chelators in food systems are ethylenediaminetetraacetic acid (EDTA), citric acid and molecules containing phosphate groups.

Next listed is the singlet oxygen quenchers which act by interaction with activated oxygen species and thus bringing them back to their ground state. In this way the oxygen quenchers have a stabilizing effect, because as described in section 1.3 the ground state oxygen is spin forbidden to perform radicalization of PUFAs (Frankel, 2005). Examples of antioxidants with singlet oxygen quenching effects are the carotenoids. The carotenoids are a highly diverse group consisting of over 600 different compounds. The singlet oxygen quenching by carotenoids can be both chemical and physical. In the chemical reaction the oxygen species react with double bonds of the carotenoids causing carotenoid decomposition. However, in the physical

quenching reactions, often found more effective, the singlet oxygen is inactivated through transfer of energy from the oxygen to the carotenoid. This results in a ground state triplet oxygen and an excited carotenoid molecule. The excited carotenoid is brought back to the ground state energy level through vibrations and rotational interaction with the surrounding solvent (Damodaran et al., 2008).

The antioxidants functioning as synergists or through regeneration act by reinforcing the activity of other antioxidants present in the substrate, and in some cases make them able to performed their antioxidant activities again (Tsao, 2015). For instance have ascorbyl palmitate and ascorbic acid been found to function as synergists for α-tocopherol by regenerating the parent α-tocopherol from the α-tocopherol radical (Kamal-Eldin & Budilarto, 2015).

The reducing agents are compounds able to donate an electron to other oxidizable compounds. These agents, for instance thioethers, can convert hydroperoxide into more stable compounds through an non-radical pathway (Pokorný, 2007). In this way the hydroperoxides are inhibited from reacting with transition metals to form free radicals which may proceed in the propagation of the lipid oxidation.

The last class listed are the enzyme inhibitors. These are secondary antioxidants which inhibit enzymes of oxidizing properties (Tsao, 2015). Despite the fact that antioxidants often are classified into a specific group by how they perform their action, it should be mentioned that it does exist antioxidants able to exert more than one type of action (Tsao, 2015). In this case, the action which will dominate in a particular food system are dependent on the surrounding conditions, both the chemical composition and the kinetic traits of the oxidation reaction (Frankel & Meyer, 2000).

1.3.1 Considerations regarding the selection of antioxidant additives

When choosing what antioxidant to add in a food product to protect it most efficiently against lipid oxidation several factors must be taken into consideration. The antioxidants found to be most effective in a substrate depends on various factors. First, the product should be evaluated to identify what prooxidants are present and which are of the greatest concern regarding the oxidative stability. Other factors such as storage conditions, which potentially could promote the lipid oxidation of the product should also be carefully evaluated. When this evaluation is completed it becomes possible to determine the antioxidants of highest relevance for protection of the product at hand. For instance, when adding antioxidants in bulk fish oil the most relevant antioxidant has been shown to be the free radical scavengers. In addition, also metal chelators

and quenchers of reactive oxygen species are observed to perform significant effects in preserving the quality of these oils (Mozuraityte et al., 2016).

Other important considerations to make are the outcome of synergistic interactions. As already mentioned more than one antioxidant are often added to a food system. This is done to optimize the protection against lipid oxidation and the total antioxidant capacity of a food system is defined as the net effect of all the antioxidants present (Tsao, 2015). Adding more than one antioxidant not only makes it possible to take advantages of their different actions, but also to achieve synergistic effects. A synergistic effect is defined by Tsao as the case when the effect of two or more antioxidant added together is greater than the sum of their individual effects when added separately (Tsao, 2015). The benefits of achieving a synergistic effect is of course the prolonged delay of lipid oxidation, but also the fact that it allows for the use of lower antioxidant concentrations. The use of lower antioxidant concentrations contributes by making the process more cost efficient, but it also has a health perspective (Tsao, 2015). Both synthetic and natural antioxidants are used in preservation of product quality. In later years, a growing concern has been expressed about the use of synthetic antioxidants in food. Several studies have been published suggesting carcinogenic effect caused by high concentrations of synthetic antioxidants such as BHA and BHT in animal trials (Makahleh, Saad, & Bari, 2015). For that reason the maximum permitted levels of these antioxidants in for instance fish oils are set by the Council Directive No 95/2/EC to 200 mg/kg (200 ppm) (EFSA, 2011). However, by taking advantage of the synergistic relationships which exists between different antioxidants, synthetic antioxidants may still be exploited efficiently, despite reduction in the concentrations added.

However, the effect caused by adding more than one antioxidant to a food system should be carefully investigated considering that the opposite of a synergistic effect, an antagonistic effect, is also a possible outcome. An antagonistic effect is the result when the effect of two or more antioxidants added together is less than the sum of their individual effects when added separately (Tsao, 2015). An antagonistic effect can be avoided by achieving knowledge about the different antioxidants and how they interact. All considerations featured in this section should also be applied for the endogenous antioxidants potentially present in the food matrix of the product.

Additional considerations regarding the use of antioxidants are solubility considerations, thermal stability and as already mentioned quantity restrictions. In what quantities an antioxidant can be added to a product is regulated by the Codex Alimentarius Commission in the Codex General Standard for Food Additives (EFSA, 2011). Knowledge about the solubility

of the antioxidant and how it distributes in the product is important to be able to ensure sufficient protection. The same goes for thermal stability. Since not all antioxidants are shown to be stable in elevated temperatures, they should be carefully selected for each application. Purity, concentration of active components, physical state and addition procedures are also factors which influence the efficiency of an antioxidant in a food system (Mozuraityte et al., 2016).

1.4 Evaluation of oxidation status

A variety of methods are developed to monitor and evaluate the state of oxidation for a given oxidizable substrate such as fish oils. Unfortunately, a standardized method able to evaluate all aspects concerning the oxidative quality and stability in any given substrate does not exist. For that reason, the methods must be carefully selected for each application. Today, the different methods available for evaluation of lipid oxidation in food systems can be classified according to what they measure. This can be the amount of oxygen absorbed, the loss of an initial substrate, the formation of free radicals or the formation of primary and secondary oxidation products (Shahidi, 2005).

The most conventional methods used for evaluation of lipid oxidation in the food industry includes peroxide value (PV), anisidine value (AV) and thiobarbituric acid reactive substances (TBARS) assay. These methods are all based on quantification of specific lipid oxidation products, or groups of products, and are performed by chemical analysis. The measurements are often used as quality indicators when evaluating the oxidative quality of a substrate. Less conventional are the accelerated oxidation tests, even though heating of oil followed by periodically testing of weight increase is one of the oldest methods used for evaluation of oxidative stability (Shahidi, 2005). These methods are time consuming and today more often automatic instruments are used when performing accelerated oxidation tests (Comandini, Verardo, Maiocchi, & Caboni, 2009). An example is determination of the oxidative stability index (OSI) which, as the Schaal oven test, is performed to evaluate oxidative stability. The method is performed by measuring the conductivity caused by volatile acidic secondary oxidation products in water. All methods are described in more detail in the following sections.

1.4.1 Peroxide value (PV) by iodometric titration

The peroxide value (PV) is a quantitative measure for the total amount of peroxides, or similar products, present in a sample and is defined as the milliequivalents of peroxide per kilogram of sample (mEq/kg) (O'Keefe & Pike, 2010). A PV measurement is one of the most conventional indicators used to evaluate the oxidative quality of fats and oils. There are several ways to measure the PV of a sample and the most frequently used methods are ferric ion complex spectrophotometry, infrared spectroscopy and iodometric titration (Shahidi, 2005).

The iodometric titration is based on a redox reaction between a saturated solution of potassium iodine (KI) and the peroxides in the oil sample. The peroxides present will oxidize the iodine ions (I^-) to iodine (I_2) (Equation 1.4.1.1).

$$ROOH + 2H^+ + 2KI \Rightarrow I_2 + ROH + H_2O + 2K^+ \quad (1.4.1.1)$$

In step two the solution is titrated against a standardized solution of sodium thiosulfate ($Na_2S_2O_3$) (Equation 1.4.1.2). The endpoint of titration is traditionally determined by use of a starch indicator, but today potentiometric endpoint determination is more common. The latter is performed by use of a platinum detection electrode which measures the amounts of liberated iodine electrochemically (Hara & Totani, 1988).

$$I_2 + 2Na_2S_2O_3 \Rightarrow Na_2S_2O_6 + 2NaI \quad (1.4.1.2)$$

Even though the use of iodometric titration for evaluation of oxidative quality is common, the method has several limitations. After the induction phase of lipid oxidation, the peroxides are formed in a high rate, but as the oxidation proceeds the rate of decomposition will surpass the rate of formation. For that reason, a low detected PV can both reflect a sample in the initial stage of the oxidation or a highly oxidized sample where the primary products are already been decomposed to secondary oxidation products. This limitation can be solved by measuring the PV over time, or by simultaneously measure the amounts of secondary oxidation products. Other main limitations of the method is that liberated iodine can be absorbed at the unsaturated bonds of fatty acids and thus result in an underestimated PV result. The analysis can also lead to overestimated results by oxygen present in the substrate causing erroneous liberation of iodine from the iodine potassium complex (Shahidi, 2005).

Iodometric titration is also relatively insensitive with a lower detection limit of 0.5 mEq/kg oil (Damodaran et al., 2008). It is a time-consuming method, which creates substantial volumes of waste and have several reaction variables, such as temperature, reaction time, sample weight and speed of stirring in which can give raise to incorrect results if not performed with high accuracy (Shahidi, 2005). Another disadvantage is the need for large sample sizes, which in some cases can be as high as 5 grams and makes the method only practical for isolated or bulk fats and oils (Damodaran et al., 2008; O'Keefe & Pike, 2010). However, despite these limitations the PV method is still one of the most applied methods for evaluating the oxidative quality of fats and oils.

1.4.2 *p*-Anisidine value (AV)

The *p*-anisidine value (AV) is also one of the more conventional oxidation parameters used in the food industry to evaluate the oxidative quality of a substrate. The method measures the quantity of aldehydes (mainly 2-alkenals and 2,4-alkadienals) present in the sample. The reaction is based on the interaction between *p*-methoxyaniline and aldehydes under acidic conditions. The reaction results in a yellow coloured Schiff base compound with absorbance at 350nm which are measured spectrophotometrically (Figure 1.4.2.1) (Shahidi, 2005). After quantification, the AV is calculated and given as 100 times the absorbance of 1g lipid dissolved in 100 ml isooctane (O'Keefe & Pike, 2010).

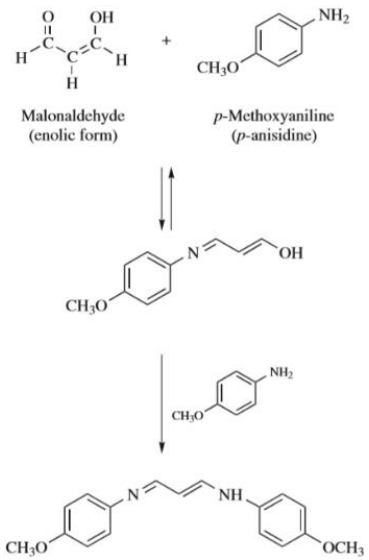

Figure 1.4.2.1: An example of the reaction taking place between an alkenal (malonaldehyde) and the reagent *p*-methoxyaniline during the AV assay, resulting in a Schiff base compound of yellow colour (Shahidi, 2005).

The AV method is favourable because it is simple to perform, also no elevated temperatures possibly leading to increased hydroperoxide decomposition is necessary. The method is also beneficial when evaluating the state of oxidation because it is more sensitive for unsaturated aldehydes compared to saturated. The reason for this is that the coloured compound resulting from the reaction with the unsaturated aldehydes have a stronger absorbance at 350nm. Unfortunately, the AV method also have some limitations. It has been criticized for having low sensitivity. Some indications have also been made that AV measurements are not comparable between different types of substrates, for instance oils of different initial PUFA concentration (Shahidi, 2005). It should also be mentioned that due to the toxicity of the *p*-methoxyaniline, great caution must be executed when performing the method.

1.4.3 Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay is one of the more conventional methods for evaluation of secondary oxidation products and was first described by Kohn and Liversedge which used the method to study lipid oxidation in meat (Kohn & Liversedge, 1944). The method is based on the reaction between secondary oxidation products, mainly malonaldehyde (MA) and malonaldehyde-like products, with thiobarbituric acid (TBA) (O'Keefe & Pike, 2010). Since MA is not the only compound reacting with TBA the group of compounds is often referred to as thiobarbituric acid reactive substances (TBARS).

The reaction of the TBARS assay is a condensation between two molecules of TBA and one molecule of MA (or a similar compound) where two molecules of water is eliminated (Figure 1.4.3.1). The reaction results in formation of a red coloured compound which can be measured spectrophotometrically at its absorption maximum, 530-535 nm (Fernández, Pérez-Álvarez, & Fernández-López, 1997; Shahidi, 2005). The results of the TBARS assay is expressed in μmol TBARS/g.

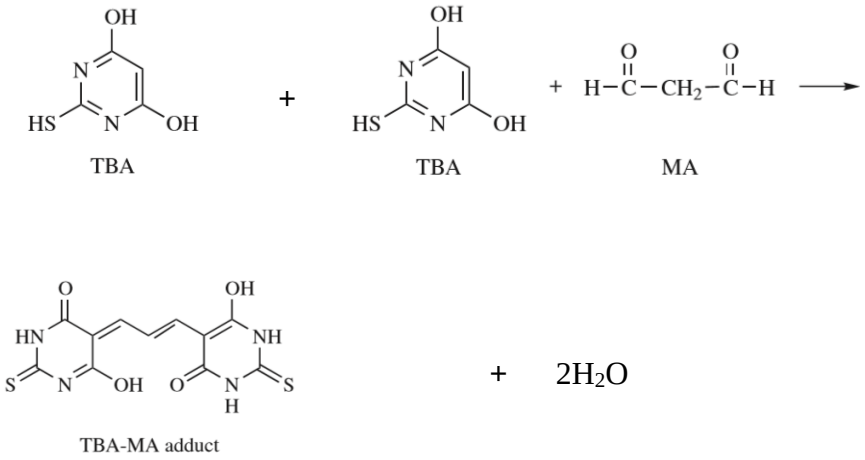

Figure 1.4.3.1: The condensation reaction between thiobarbituric acid (TBA) and a malonaldehyde (MA) results in a TBA-MA red coloured adduct which can be measured spectrophotometrically (Shahidi, 2005).

Although the TBARS assay is widely utilized for evaluation of lipid oxidation, it is well-known to have some limitations regarding specificity and sensitivity. The TBA is shown to be selective for compounds other than MA, including other aldehydes, carbohydrates, amino acids and nucleic acids (Salih, Smith, Price, & Dawson, 1987). This may lead to overestimated results caused by other TBA adducts absorbing light at wavelengths overlapping the red peak absorbed by MA (Shahidi, 2005). Underestimated results may also occur by MA forming linear or cyclical Schiff bases with the amino acids lysine and arginine originating from proteins and in this way be hindered from reacting with the TBA reagent. Other limitations regarding the TBARS assay is the sensitivity towards variations in reaction conditions. Variations to conditions such as heat exposure time, temperature, pH and concentration of TBA solutions have been shown to significantly influence the results of the TBARS assay (Barriuso, Astiasarán, & Ansorena, 2013). To optimize the procedure and improve the specificity and sensitivity several improvements have been proposed including reducing the temperature of the heating step, adding of antioxidants before running the test and performing the test on a extraction of MA (Shahidi, 2005). Alternatively, a more accurate method for determination of malonaldehydes such as high pressure liquid chromatography (HPLC) analysis can be used (O'Keefe & Pike, 2010). However, despite the limitations and more accurate methods available, the TBARS assay is cherished for its simplicity and is still a widely utilized and preferred quality indicator for fats and oils (Barriuso et al., 2013).

1.4.4 The Schaal oven test

The Schaal oven test is an accelerated oxidation test. In the accelerated oxidation tests the samples are exposed to elevated temperatures in the presence of oxygen to accelerate the lipid oxidation rate. The Schaal oven method is used to evaluate the oxidative stability of samples and are based on the principle that when fats and oils are oxidised they increase in weight as a result of oxygen binding (Mozuraityte, Kristinova, Standal, Evensen, & Rustad, 2017).

Today the Schaal oven test is a recommended practice of the AOCS (Appendix A). The procedure involves placing oil samples of known weight in a heat cabinet at elevated temperature. The temperature is recommended to be above room temperature, but below 80 degrees (preferably 60 degrees). These specific temperatures are recommended because the oxidation process is expected to evolve approximately as it would do in room temperature only faster. The test should be performed in darkness to avoid the impact of light induced lipid oxidation, the initial quality of the oil should be high and the surface to volume ratio should be kept equal and constant for all samples (O'Keefe & Pike, 2010). By limiting the factors other than heat, that could potentially influence the lipid oxidation, it becomes possible to assess the differences in weight increase trends for different samples. How fast the weight increase evolves in a sample reflects the oxidative stability of the sample and is often expressed as the induction phase which is the time before an observed maximum change in the rate of the weight increase. To evaluate this, the weight increase measurements is plotted as the function of time and the resulting graphs are compared to assess differences in induction phases between different samples (Figure 2.5.2.1). If the weight increase occurs fast it suggest the sample to have a low oxidative stability compared to samples where the induction phase is observed to last longer.

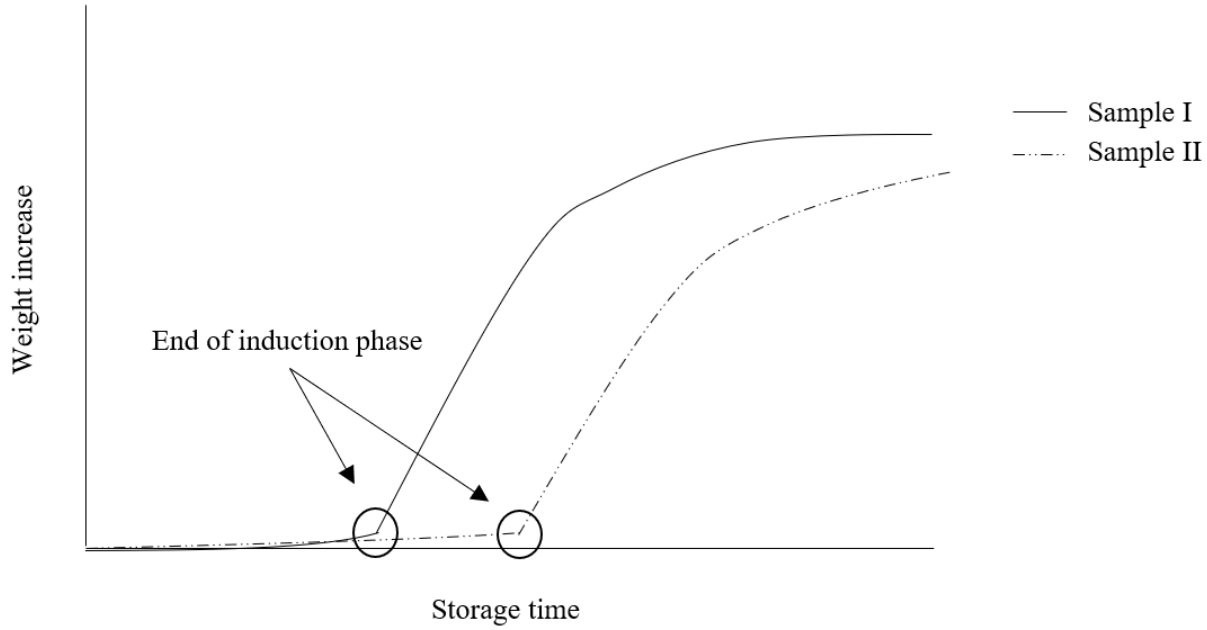

Figure 2.5.2.1: The figure illustrates how the results of a Schaal oven test are plotted and how a difference in induction phase between two samples is observed.

The simplicity of the Schaal oven test is a huge advantage and the requirements for equipment is low. However, the method also has some limitations. The foremost limitation of the method is the assumption made that the reaction rate observed for the samples in the test conditions are the same as it would be during normal storage conditions (O'Keefe & Pike, 2010). Another limitation is that the observed changes in mass, also can originate from other volatiles present in the sample, making the changes in weight reflect more than just the oxygen consumption (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). To counteract this the sample could be preheated in an inert atmosphere before performing the test. However, since heat is an initiator of lipid oxidation this could potentially lead to further complications. The surface to volume ratio is, as mentioned, a critical factor of the Schaal oven test. Since the lipid oxidation reaction is oxygen dependent the oxidation rate will decrease, as the volume-to-surface ratio increase. Variations in this factor across studies are limiting the comparability of the results. Other factors to keep in mind are the importance of cleaning the equipment between runs to ensure there is no contaminants present such as transition metal ions and oxidation products from previous runs (O'Keefe & Pike, 2010).

1.4.5 Oxidation stability index (OSI)

The oxidation stability index (OSI) is an accelerated oxidation test used to evaluate the oxidative stability of oil and fat samples. The method is performed by blowing purified air through the sample while keeping the sample at elevated temperature, usually at 110-130 degrees and in this way accelerating the oxidation (O'Keefe & Pike, 2010). Volatile acids formed as secondary lipid oxidation products in the samples are passed on with the blowing air to a container of deionized water. The conductivity of the water is continuously measured and as the samples becomes oxidized, more volatile compounds are formed and transferred to the water where the conductivity increases. The conductivity measurements can be plotted as a function of time to visualize the progress of the lipid oxidation. The OSI value of the sample is defined as the point where maximum change is observed for the increase in conductivity. This change reflects a change in the lipid oxidation rate and marks the end of the induction phase (Shahidi, 2005). The time it takes for a sample to reach this point reflects the oxidative stability of the sample. A longer induction phase would normally correlate with a higher oxidative stability (O'Keefe & Pike, 2010).

The accelerated oxidation tests are helpful because they can give information concerning the shelf life of a substrate, which under actual storage conditions would take months or even years to achieve. The drawback of these test is the possibility that the test does not sufficiently reflects the true development of the lipid oxidation that would take place during normal storage conditions. It is for that reason important to be critical to the results and it has been suggested to perform other methods simultaneously to validate the observations. Methods which can be used to validate are PV, AV and TBARS assay which directly quantify the amounts of lipid oxidation products.

If compared to the Schaal oven storage test, the OSI is less time consuming and gives faster results. However, it is important to take into consideration that the temperatures are often higher, and the oxygen exposure are more intense. Due to this the results may differ more from the true development observed in normal storage conditions. The deviant results is suggested to be caused by the formation of compounds at higher temperatures which would not necessarily be formed during normal storage conditions (O'Keefe & Pike, 2010). The OSI method is also limited to samples in liquid form and are not optimal for samples containing large amounts of water since such samples would dehydrated rapidly due to the temperatures normally utilized during the OSI procedure (Mozuraityte et al., 2017).

1.5 Aim of thesis

The parameters used as indicators for oxidative quality and stability in the food industry today are many. The most conventional methods for evaluation of quality are peroxide value (PV), anisidine value (AV) and thiobarbituric acid reactive substances (TBARS) assay. PV measures the primary oxidation products, hydroperoxides, while AV and TBARS measures secondary oxidation products. Less conventional methods available are the accelerated oxidations tests such as Schaal oven test and oxidative stability index (OSI). While PV, AV and TBARS assay provides a value which can be used to reflect on the here-and-now quality of the oil sample, the accelerated oxidation tests are used to evaluate the oxidative stability by determining the length of the induction phase. The aim of the work performed in this thesis have been to study the lipid oxidation in fish oils by use of the conventional methods PV and TBARS assay and the accelerated oxidation test, Schaal oven test and OSI.

In the first experiment the aim was to study the differences in lipid oxidation for a fish oil exposed to five different storage temperatures using PV determination, TBARS assay and the Schaal oven test. The oil utilized in this experiment was a refined cod liver oil without addition of an antioxidant additive. In the second experiment performed, the aim was to study the stabilizing effect of various antioxidant additives when added in different fish oil samples. This was performed by use of the accelerated oxidation test, Schaal oven test. In this experiment five different fish oils, described in section 2.1, and six different antioxidant additives, described in section 2.2, was utilized. The last aim of this thesis was to compare some of the results from the Schaal oven test with results from OSI to evaluate whether the observed order of antioxidant potency would be the same in both tests.

2. Materials and methods

2.1 The fish oils

The fish oil utilized in the work with this master thesis were donated from an unknown fish oil supplier in Norway. The oils are R&D samples and it should be emphasized that they are not representative for the final products sold by the supplying producer. None of the oils had been added with antioxidants when received from the supplier. Known characteristics of each fish oil, together with the sample code used further in this thesis, are given in table 2.1.1. The fish oils were stored at -80°C in containers protected from light. After each sampling, the oils were flushed with nitrogen and returned to the freezer to limit undesired lipid oxidation.

Table 2.1.1: An overview of the fish oils used in the experimental work with this thesis. The table gives the known characteristics of each fish oil together with the sample codes used to describe the fish oils later in this thesis.

Sample code	Characteristics
TRAN	Refined cod liver oil.
18/12-S	18/12 - Anchovy oil. Treatments: neutralisation and stripping
18/12-B	18/12 Anchovy oil. Treatments: neutralisation, stripping, winterisation, and bleaching.
TG60	Anchovy oil, 60% of the fatty acids present as triglycerides. Treatments: neutralisation, stripping, transesterification to EE, concentrated by distillation, transesterification back to TG, winterisation, and bleaching.
TG90	Anchovy oil, 90% of the fatty acids present as triglycerides. Treatments: neutralisation, stripping, transesterification to EE, concentrated by distillation, transesterification back to TG, distillation of excess EE, winterisation, bleaching and deodorisation

2.2 The antioxidants

The antioxidant additives used in the work with this thesis were supplied by an unknown producer. In what concentrations the antioxidants were utilized, together with the alias used further in this thesis, are listed in table 2.2.1.

Table 2.2.1: An overview of the antioxidants used in the experimental work with this thesis. The table gives the known functioning components of the antioxidant additives together with an alias representing the antioxidant additive when described later in this thesis.

Antioxidant alias	**Functioning antioxidants**	**Concentrations used in analysis**
Mixed tocopherols	α-, β-, γ- and δ-tocopherols	1000 ppm
α-tocopherol	α-tocopherol	1000 ppm
A/L/T mixture	Ascorbyl palmitate, lecithin, and α-tocopherol	1000 ppm
AP	Ascorbyl palmitate	1000 ppm
Rosemary extract	Carnosic acid, carnosol and rosmarinic acid	150 ppm
BHA	Butylated hydroxyanisole	1000 ppm

2.3 Reagents and solvents

The following reagents and solvents were utilized in the experiments performed in the work with this thesis. The supplying producer is listed in the parentheses. TBARS assay: acetic acid (Merck, Germany), distilled water, sodium sulphite (Merck, Germany), 1,1,3,3-tetraethoxypropane (Sigma Aldrich, USA), 2-thiobarbituric acid (Sigma Aldrich, USA), trichloroacetic acid (Merck, Germany). PV: acetic acid (Merck, Germany), chloroform (VWR Chemicals, France), distilled water, potassium iodine (Merck, Germany), sodium thiosulfate (Merck Germany).

2.4 Experimental design

2.4.1 The TRAN oil experimental design

In the first experiment of this thesis only the refined cod liver oil without addition of antioxidant (TRAN) was used. 8-10 parallels of the fish oil were stored at each storage temperature (10, 22.5, 30, 40 and 50 degrees). For every storage temperature the experimental design presented in figure 2.4.1.1 was implemented.

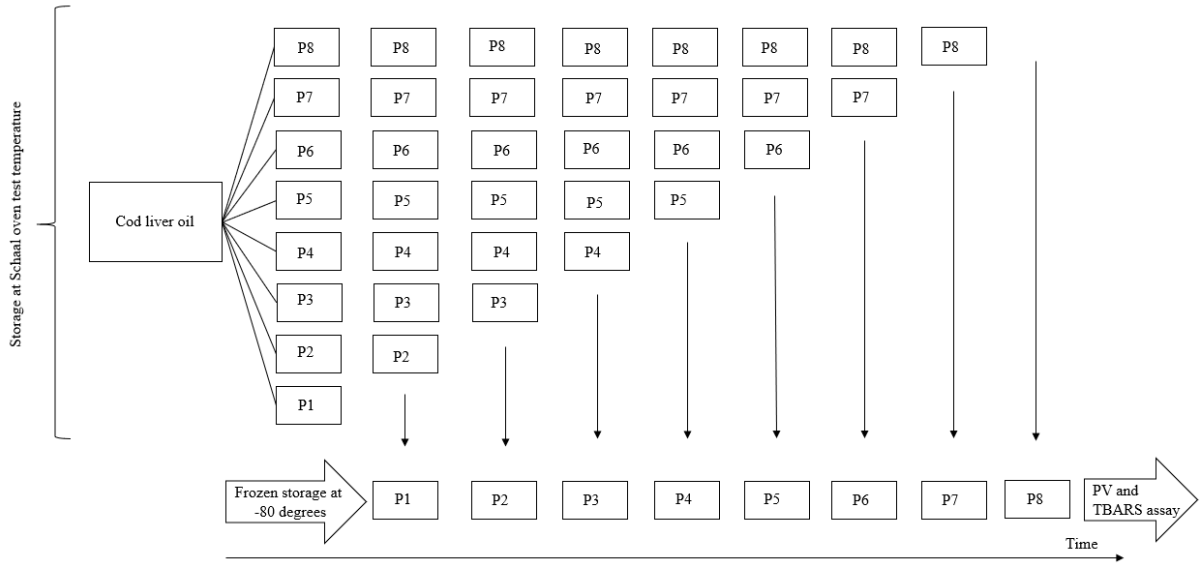

Figure 2.4.1.1: For each of the five storage temperatures utilized in the TRAN oil experiment, the illustrated experimental design was performed. P1-P8 are the parallels initially placed in the heat cabinet and the flowchart show how one parallel was removed from the experiment each day and frozen down pending further analysis.

The parallels of a specific storage temperature were weighed twice a day and each day during weighing one of the parallels were taken out of the experiment, transferred to a sample tube and frozen down to -80 degrees. The Schaal oven test was completed when all parallels was frozen down. By using this type of experimental design, the parallels of a specific storage temperature were retained in the oven for different amounts of time. In addition to monitoring the weight increase, this experimental design thus enabled monitoring of the oxidation product formation through performing PV and TBARS assay for all parallels.

2.4.2 The antioxidant experimental design

In the antioxidant experiment, five different fish oils were utilized. The fish oils were TRAN, 18/12-S, 18/12-B, TG60 and TG90. The oils were described in more detail in section 2.1. For each fish oil seven different samples were prepared: one control without addition of antioxidant additive and six samples each with a different antioxidant additive. All additives were added to the fish oil samples at a concentration of 1000 ppm, except the additive BHA which was added at a concentration of 150 ppm. Information about the different antioxidant additives were given in section 2.2. The preparation of these samples resulted in 29 individual samples which are presented in table 2.4.2.1.

Table 2.4.2.1: An overview of the different samples prepared for the antioxidant experiments. The samples marked with a "-" were excluded from the experiments because of poor antioxidant solubility in the particular fish oil.

	TRAN	**18/12-S**	**18/12-B**	**TG60**	**TG90**
No AOX	TRAN No AOX	18/12-S No AOX	18/12-B No AOX	TG60 No AOX	TG90 No AOX
Mixed toco.	TRAN + Mixed toco	18/12-S + Mixed toco	18/12-B + Mixed toco	TG60 + Mixed toco	TG90 + Mixed toco
α-tocopherol	TRAN + α-toco	18/12-S + α-toco	18/12-B + α-toco	TG60 + α-toco	TG90 + α-toco
A/L/T	TRAN + A/L/T	18/12-S + A/L/T	18/12-B + A/L/T	-	-
AP	TRAN + AP	-	-	-	-
Rosemary	TRAN + Rosemary	18/12-S + Rosemary	18/12-B + Rosemary	TG60 + Rosemary	TG90 + Rosemary
BHA	TRAN + BHA	18/12-S + BHA	18/12-B + BHA	TG60 + BHA	TG90 + BHA

For all samples listed in table 2.4.2.1 a Schaal oven test with 3-4 parallels was performed at 50 degrees. In these experiments the aim did not include evaluation of formed oxidation products and for that reason all parallels were retained in the heat cabinet until the end of the experiment. This is implied in the flowchart in figure 2.4.2.1. For how long time the weight increase was monitored for the different samples varied, and was dependent on the weight increase rate of each individual sample.

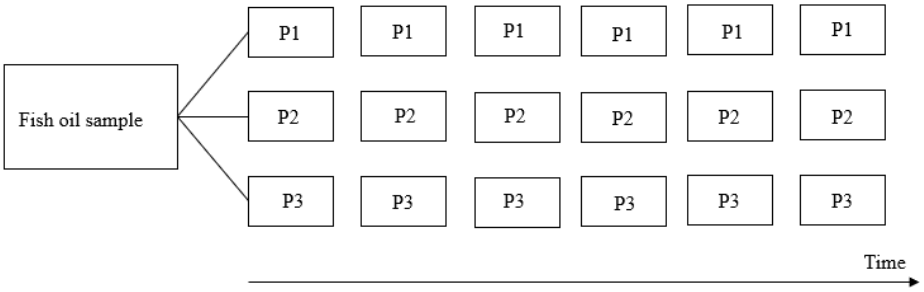

Figure 2.4.2.1: For each fish oil sample 3-4 parallels (P1-P3) was stored at 50 degrees while the weight increase was continously monitored. The flowchart illustrates how all parallel of the fish sample was kept in the heat cabinet throughut the experiment.

2.5 Analytical methods

The peroxide value (PV) by iodometric titration, the thiobarbituric reactive substances (TBARS) assay and the Schaal oven storage tests was performed at the Department of Biotechnology and Food Science, NTNU. The determination of fatty acid composition by Gas chromatography and the Oxidative stability index (OSI) were performed at SINTEF Ocean, Trondheim.

2.5.1 Determination of fatty acid composition by Gas chromatography (GC)

The fatty acid (FA) composition of the fish oils was determined by Agilent Technologies 7890A gas chromatograph and flame ionisation detector (FID). The methylation step and the GC-FID analysis were performed as described by Kristinova et al. (Kristinova, Mozuraityte, Aaneby, Storrø, & Rustad, 2014), while the identification of the different methyl esters was performed by comparing their retention times to those of the reference solution (Nu-Chek Prep Inc.) analysed under identical conditions. The result of each fatty acid are presented as a percentage of the total fatty acid content in the sample. For each fish oil sample two parallels were tested. The procedure was performed by Merethe Selnes at SINTEF Ocean.

2.5.2 The Schaal oven test

The Schaal oven test was performed following the recommendations given in the AOCS official recommended practice Cg 5-97 (Appendix A). Approximately 7 grams of fish oil per parallel was weighed and placed in glass petri dishes of equal size (diameter = 7.5 cm) to provide equal and constant surface to volume ratio. The petri dishes were placed without coverage in a dark heat cabinet of a specific temperature. The parallels were periodically taken out of the cabinet and weighed to continuously monitor the weight increase.

The TRAN oil experiment

In the first experiment only the refined cod liver oil without addition of antioxidants (TRAN) was used, and the Schaal oven test was performed for the fish oil at five different storage temperatures (10, 22.5, 30, 40 and 50 degrees). For each storage temperature 8-10 parallels were initially placed for storage, but during the experiment parallels were periodically taken out of the experiment and frozen to -80 degrees pending further analysis. The experimental design of the TRAN oil experiment was explained in more detail in section 2.4.1.

From the weight increase measurements collected during the Schaal oven test the mean weight increase at each weighing was calculated and converted to the unit of g/100g fish oil. Then the weight increase measurements for each storage temperature were plotted as a function of time to observe the length of the induction phase. This was done to evaluate how the oxidative stability of the oil varied between the different storage temperatures. Details about how the induction phase was observed was described in more detail in section 1.4.4. Further, PV and TBARS assay were performed for all parallel of each storage temperature to evaluate the development of primary and secondary oxidation products during the time of storage.

The antioxidant experiments

In the antioxidant experiment five different fish oils were utilized. The fish oils were TRAN, 18/12-S, 18/12-B, TG60 and TG90 which are described in section 2.1. For each type of fish oil seven different samples were attempted to prepare, one without antioxidant additive and six added with an antioxidant additive. However, not all additives dissolved in all the five different fish oils. The different antioxidant additives utilized and in what concentrations was presented in table 2.2.1 of section 2.2, and an overview of the different samples prepared, together with the experimental design was presented in section 2.4.2.

The antioxidants were dissolved in the oil samples by first weighing the right amount of antioxidant additive in a 250 mL glass beaker. Then, 100 mL of fish oil was transferred to the beaker. Stirring was performed with a magnet stirrer at a certain speed for approximately 6-10 minutes. If an antioxidant additive did not dissolve readily the sample was excluded from proceeding in the experiment. For each sample 3-4 parallels were prepared and stored at 50 degrees while continuously monitoring the weight increase. In this experiment no parallels were removed from the experiment and frozen down during the storage period. The length of the Schaal oven test varied between samples and was adjusted according to how fast the weight increase evolved. Normally the test was terminated when the weight increase was observed to cease. The weight increase measurements collected for each sample were plotted as a function of time and the induction phase for each sample was evaluated as described in section 1.4.4. This was done to assess the stabilizing effects of each antioxidant additive in the different fish oils.

2.5.2 Oxidation stability index (OSI)

The oxidative stability index was determined for a selection of samples using an oxidative stability instrument (OSI-24) delivered by ULTRA Scientific. The procedure was performed by Revilija Mozuraityte at SINTEF Ocean, Trondheim.

2.5.3 Peroxide value (PV) by iodometric titration

The peroxide value (PV) of the samples were determined by iodometric titration. The analysis was performed as described in the AOCS Official method Cd 8-53 with some alterations (Appendix B). The titration was performed using an automatic titrator delivered by SI Analytics connected to a platinum detection electrode (Pt 62/61). Depending on the expected level of oxidation for a given sample, 0.02-1 gram of fish oil was weighed and transferred to a 150 mL glass beaker. In preparation for the titration, a mixture of 18 mL acetic acid and 12 mL chloroform was added to dissolve the fish oil. Further, 0.5 mL of saturated potassium iodide solution was added, and the beaker was covered with aluminium foil and placed for stirring in 60 seconds. After 60 seconds, 30 mL of distilled water was added, and the automatic titration was performed during continuous stirring. The titration volume of the sample was noted. The analysis was performed for 3 parallels of each sample. Before measuring the sample parallels a blank sample was determined by the same procedure, only without fish oil. For each sample parallel the PV was calculated in the unit of mEq peroxide per kg oil by the following formula:

$$\text{PV (mEq peroxide/kg oil)} = \frac{C \times (V - \text{blank}) \times 1000}{m}$$

C = titrant concentration in mol/L

V = titrant volume in mL

m = mass of oil sample in gram

2.5.5 Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay was performed as described by Ke & Woyewoda with some alterations (Ke & Woyewoda, 1979). 0.5 gram of each oil sample was dissolved in 10 mL of chloroform. Further 200 µl of the sample was transferred to kimax tubes. 5 mL of TBA work solution was added and the kimax tube was closed tightly, mixed for 15 seconds in a vortex, and incubated in water of 95 degrees for 45 minutes. After cooling in running tap water, 2.5 mL of TCA solution were added to the sample tube. Separation of the chloroform and water phase was performed by centrifugation for 10 minutes at 900G. The water phase was transferred to a 10 mm quartz (QS) cuvette and absorbance was measured at 538 nm. Distilled water was used as the reference. In case of elevated absorbance values (>900) the samples were diluted with 55% acetic acid before measuring the absorbance. The assay was performed for 3 parallels of each oil sample.

A standard curve for use in the final calculations was prepared from TEP solutions of known concentrations. This was prepared using a 0.1 mM TEP working solution. The amounts of TBARS in a sample was calculated using the following formula:

$$\mu M \text{ TBARS / gram oil} = (A-b) / (a * m * 1000)$$

A = absorbance of the oil
a = slope of the standard curve
b = intercept of the standard curve
m = amount of sample oil (gram)
1000 = conversion to µM / gram

2.6 Equipment

Table 2.6.1: List of equipment applied in the laboratory work of this study. From left the type of equipment, model and distributor are listed.

Equipment	Model	Producer
Automatic titrator	TitroLine 7000	SI Analytics
Centrifuge	Heraeus Multifuge X1R	Thermo Fisher Scientific
Detection electrode	Pt 62/61	SI Analytics
Glass quartz cuvettes	104-QS	Hellma Analytics
Gas chromatograph	7890a	Agilent Technologies
Heating bath	GD100	Grant
Heat cabinet	BD	Binder
Magnetic stirrer	MR – mini	Heidolph
Magnetic stirrer	TM235	SI Analytics
OSI	OSI-24	ULTRA Scientific
UV-spectrophotometer	G10S UV-Vis	Thermo Fisher Scientific
Vortex mixer	REAX TOP	VWR International
Weight	MS204TS/00	Mettler Toledo

2.7 Statistics

The uncertainty of all measurements presented in this thesis are given as the standard deviation (SD) of the number of parallels utilized in a given experiment. The standard deviation was calculated by the formula 2.7.1

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x-\bar{x})^2}{n-1}} \qquad (2.7.1)$$

To evaluate significant difference between measurement A and B, the uncertainty in the standard deviations of the measurements (δq) was first calculated by the formula 2.7.2.

$$\delta q = \sqrt{a^2 + b^2} \qquad (2.7.2)$$

a = SD for measurement A

b = SD for measurement B

Further a t-value was calculated by the formula 2.7.3 and used to determine the probability of measurement A being significantly different from B. This was done using a table of normal error integral and a confidence interval of 95% (Taylor, 1997).

$$t = \frac{[A-B]}{\delta q} \qquad (2.7.3)$$

Exclusion of measurements suspected to be spurious as a result of mistakes made performing the procedures were removed from the experimental data using the Chauvenet's Criterion (Taylor, 1997). All calculations were performed using the Microsoft Excel spreadsheet software.

3. Result and discussion

3.1 The fatty acid composition

The fatty acid composition is of great importance for the progression of the lipid oxidation in substrates such as fish oils. A high degree of unsaturation is often in accordance with the oil being extensively prone to lipid oxidation. To describe the variations between the oils used in the work with this thesis, the fatty acid compositions of the oils were determined by GC-FID. The results are presented in table 3.1.1. The content of each fatty acid group investigated is given as a percentage (%) of the total fatty acid content of the samples. All values are given as an average of two parallels and the uncertainty is given as standard deviation (SD). Characteristics of the different fish oils were given in section 2.1 and the complete data sets from the GC-FID analysis are enclosed in appendix C.

Table 3.1.1: An overview of the results from determination of fatty acid composition by GC-FID for the oils utilized in the work with this thesis. The values are given as a percentage (%) of total fatty acid content in the sample, together with the standard deviation (SD). All values are given as the mean of two parallels.

	TRAN		**18/12-S**		**18/12-B**		**TG60**		**TG90**	
	%	**SD**	**%**	**SD**	**%**	**SD**	**%**	**SD**	**%**	**SD**
Saturated	19.77	0.03	36.99	0.28	33.90	0.12	9.09	0	10.39	0.03
Monosaturated	52.16	0.05	26.57	1.06	28.32	0.09	19.25	0.02	19.73	0.04
Polysaturated	28.07	0.08	36.44	0.78	37.79	0.21	71.66	0.03	69.88	0.07
Omega-3	24.61	0.08	33.15	0.57	34.78	0.21	67.19	0.03	65.49	0.03
EPA	8.80	0.04	16.06	0.37	18.41	0.09	36.08	0.05	35.03	0.02
DHA	10.15	0.12	10.44	0.02	9.78	0.11	22.07	0.10	21.51	0.01

The five fish oils were all observed to have a relatively high content of PUFAs ranging from 28.07% in TRAN, 36.44% in 18/12-S, 37.79% in the 18/12-B, 69.88% in TG90 and 71.66% in TG60. Since the degree of unsaturation is of particularly high importance considering the susceptibility towards lipid oxidation, the content of PUFAs in each fish oil as a percentage of total FA content is emphasized in figure 3.1.1.

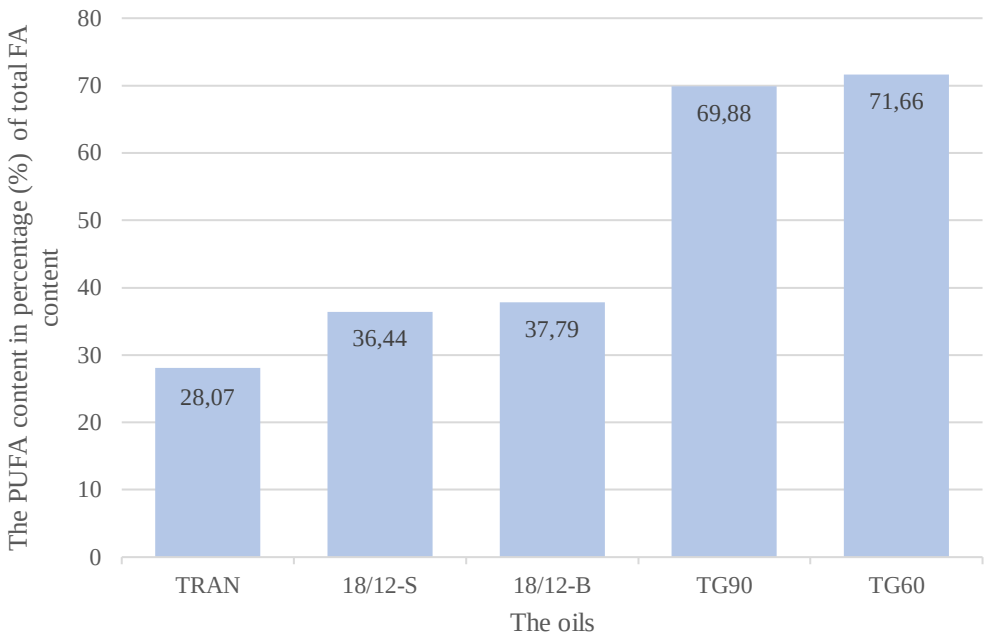

Figure 3.1.1: The results from determination of the PUFA content in percentage (%) of the total FA content in the five fish oils studied in this thesis. Each value is based on two parallels.

The TG60 and TG90 was found to have the highest content of PUFAs, which also was expected considering the samples are fish oil concentrates. The difference in PUFA content between the TG60 and TG90 oils were evaluated using formula 2.7.3 given in section 2.7 and found to be significantly different ($p<0.05$). This relation is suggested to be a result of the different treatments received by the two fish oils. While the TG90 oil received a distillation step for removal of excess ethyl esters (EE) after transesterification, the TG60 did not. During the distillation of excess EE it would be expected for some PUFAs, still in the ethyl ester form, to be removed. For that reason, this treatment step is suggested to be a contributor to the lower and significant different PUFA content observed in the TG90 fish oil compared to TG60. Additionally, the TG90 fish oil was also exposed to a deodorisation step which the TG60 fish oil was not. A deodorisation step which involves exposure to highly elevated temperatures can as supported by literature also be a contributing factor to the significantly lower PUFA content observed for the TG90 oil (Fournier et al., 2006).

The difference in PUFA content observed for the fish oil samples 18/12-S and 18/12-B was also evaluated using the formula 2.7.3 given in section 2.7. This difference was not found to be significantly different ($p>0.05$) even though the treatments received by the oils were different. In addition to neutralisation and stripping which were received by both fish oils, the 18/12-B oil was also exposed to winterisation and bleaching. It is therefore suggested that this treatment steps did not contribute to a significant change in PUFA content. However, since the winterisation step is performed with the purpose of decreasing the content of saturated fatty acids by gradually cooling the fish oil and removing fats with high melting points, it would been expected to observe an increased PUFA content in percentage of total FA for the 18/12-B fish oil after receiving this treatment. An increase was observed, but the difference was not found to be statistically significant compared to the 18/12-S fish oil PUFA content, which did not receive the winterisation treatment. The bleaching step is performed to improve colour and oxidative stability of the fish oils. The process removes oxidation products and trace metals which might have been formed during the refining of the fish oils (Breivik, 2007). However, literature describing significant change in PUFA content as a result of bleaching has not been found and supports the observations done in this experiment. The TRAN fish oil was not included in these comparisons due to lack of information regarding the treatment received by this fish oil during manufacturing.

3.2 The TRAN fish oil experiment

3.2.1 The Schaal oven test

In this experiment the Schaal oven test was performed for the same fish oil at five different temperatures. The fish oil utilized was the refined cod liver oil without addition of antioxidants (TRAN). The temperatures chosen for the experiment was 10, 22.5, 30, 40 and 50 degrees. The experiment started off with placing 8-10 parallels in each storage temperature. Twice a day the samples were weighed to monitor weight increase and periodically one parallel was taken out of the experiment and frozen down pending further analysis. The latter step was performed approximately once a day until only one parallel remained in the heat cabinet at the last weighing.

From the weight measurements collected, the mean weight increase at each weighing was calculated and converted to the unit of g/100g fish oil. Some of the samples showed a slight weight decrease the first two hours. This was assumed to be caused by trace water and other volatile compounds that were evaporated from the sample when first exposed to the elevated temperatures. For that reason, all weight increase measurements in this experiment are

calculated by applying the weight measured after 2 hours as the reference zero weight. The results are presented in figure 3.2.1.1. Each curve represents a specific storage temperature and show the development in mean weight increase as a function of time. The uncertainties of the measurements are given as standard deviation and is, together with all weight increase data, enclosed in appendix D.

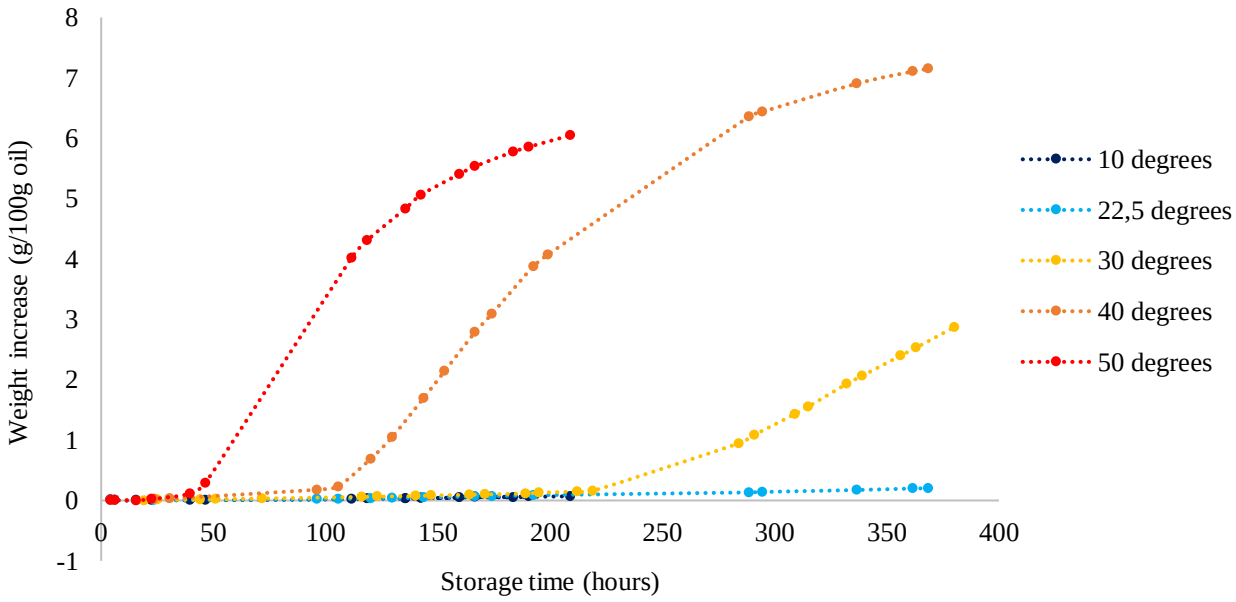

Figure 3.2.1.1: The result from the Schaal oven test performed for the TRAN fish oil in five different storage temperatures. Each curve represents a storage temperature and the weight increase results for each temperature are given in g/100g oil as a function of storage time given in hours.

As described in previous sections and supported by literature, the ambient temperature of a fat containing substrate influence the lipid oxidation rate in the substrate (Damodaran et al., 2008). The fish oils are especially prone to the lipid oxidation reactions, considering their high content of PUFAs. In this experiment the length of the induction phase was observed for storage temperatures 30, 40 and 50 degrees to be approximately 219, 105 and 40 hours, receptively (Figure 3.2.1.1). The induction phases were observed as described in section 1.4.4 and the length of the induction phase implies the oxidative stability of the fish oil sample. In this way a longer induction phase equals a higher oxidative stability. Considering this information, the resistance towards lipid oxidation in the TRAN fish oils stored at 30, 40 and 50 degrees, was found to decrease with increasing storage temperature. These results har support in literature which describes how elevated storage temperatures accelerates the rate of lipid oxidation (Damodaran et al., 2008). Similar results have also been publish by Kasbo (Kasbo, 2012).

For the TRAN fish oil samples stored at 10 and 22.5 degrees, the end of the induction phase was not observed within the time of this experiment. However, it would have been expected for these samples to reach the end of the induction phase if the storage time had been prolonged. Even though the end of the induction phase was not observed, the samples of both storage temperatures showed steady weight increase during the experiment. Weight increase data are given in appendix D.

In table 3.2.1.1 the approximate times for each sample to reach 0.05% weight increase are given. The low percentage chosen for the comparison in this experiment (0.05%) was necessary to be able to compare the samples of all five storage temperatures.

Table 3.2.1.1: The approximate times for the TRAN fish oil samples to reach 0.05% weight increase in the five storage temperatures 10, 22.5, 30, 40 and 50 degrees.

Storage temperatures (celsius)	Time to reach 0.05% weight increase (hours)
10	160
22.5	145
30	110
40	40
50	30

3.2.2 Development of oxidation products during the Schaal oven test

The parallels collected and frozen to -80 degrees during the Schaal oven tests of the TRAN fish oil samples were further analysed for content of primary and secondary oxidation products. This was performed using PV and TBARS assay. The aim was to study how the development of oxidation products differed between the five storage temperatures (10, 22.5, 30, 40 and 50 degrees).

PV results

Figure 3.2.2.1 and 3.2.2.2 presents the result of the PV measurements performed for the TRAN fish oil samples. Each curve represents a storage temperature and the PV measurements are given in mEq per kg oil as a function of time given in hours. Most PV measurements are given as the mean of three parallels, with a few exceptions due to technical issues. The uncertainty of the measurements is given as the standard deviation and is represented in the figures as error bars. All data relevant for the PV calculations are enclosed in appendix E.

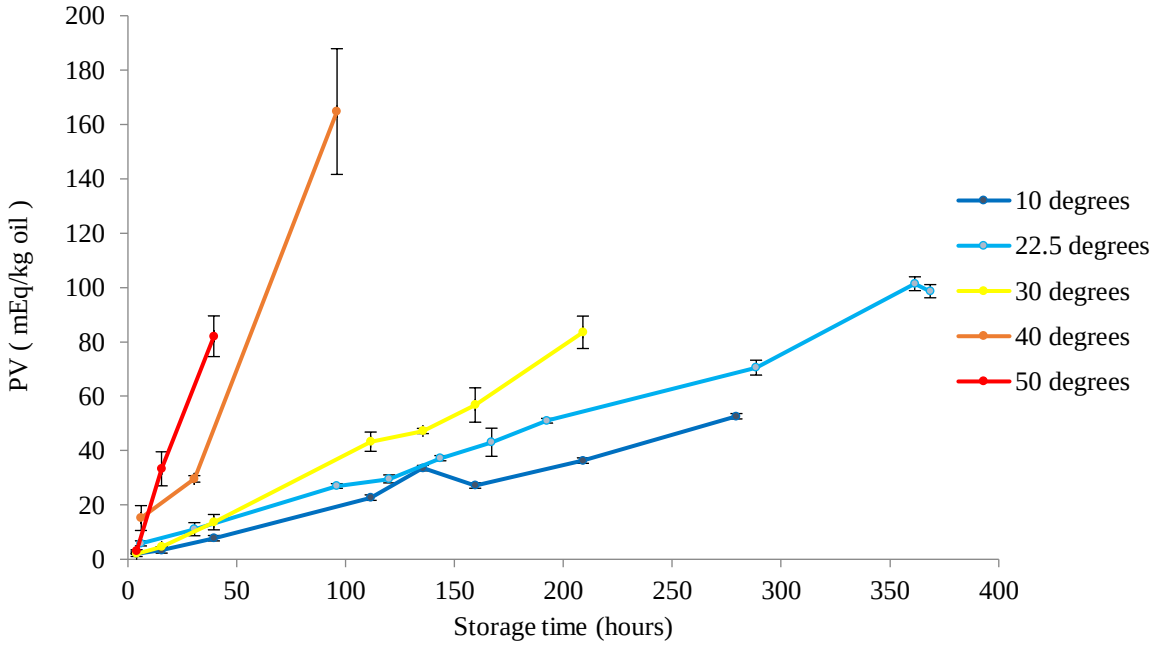

Figure 3.2.2.1: The result of PV measurements for the TRAN fish oil parallels collected during the run of Schaal oven test. Each curve represents a storage temperature (10, 22.5, 30, 40 and 50 degrees) and the PV is given as mEq/kg oil as a function of storage time given in hours. The error bars show the standard deviation of the measurements.

To be able to compare the development of primary oxidation products for all five temperatures in the same figure only the three first measurements for the storage temperatures 40 and 50 degrees are shown in figure 3.2.2.1. This was necessary to be able to visualize the development for all temperatures within the same figure. A figure showing all measurements for all temperatures are discussed later in this section. The PV is observed to develop slower in the oils stored at the three lower temperatures, 10, 22.5 and 30 degrees. Among these, the highest rate was observed for the fish oil samples stored at 30 degrees, followed by the fish oil samples stored at 22.5 degrees and the lowest rate was observed in the fish oil samples stored at 10 degrees. These results are supported in literature, where the rate of which primary oxidation products are formed are found to increase with increasing temperature exposure (Damodaran et al., 2008).

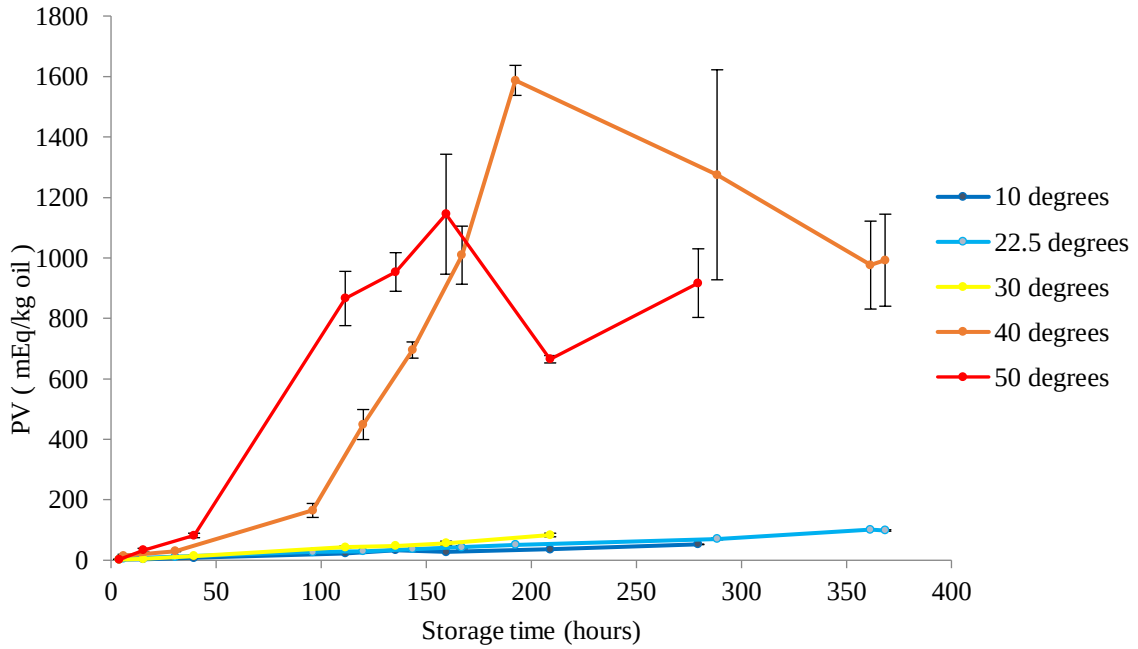

Figure 3.2.2.2: PV results for the TRAN fish oil samples stored at the five storage temperatures 10, 22.5, 30, 40 and 50 degrees. Each curve represents a storage temperature and the PV measurements are given as mEq/kg oil as a function of storage time in hours. The error bars show the standard deviation of the measurements.

Figure 3.2.2.2 show the mean for all PV measurements performed for the TRAN fish oil samples. The fish oil samples stored at 40 and 50 degrees both starts off with the PV increasing at a lower rate before eventually reaching a point where the PV starts to increase nearly exponentially. The fish oil sample stored at 50 degrees was found to reach this point before the fish oil sample stored at 40 degrees. This observation as already mentioned correlates well with the literature stating that exposure to higher temperatures accelerates the development of primary oxidation products in fish oils. Further, the exponential increase in PV for the sample stored at 40 degrees was found to start at approximately 100 hours of storage, which correlates well with the time when the end of the induction phase was observed for this temperature in the Schaal oven test. The exponential increase in PV for the sample stored at 50 degrees was found to start at approximately 40 hours of storage, this also correlates well with the results observed from the Schaal oven test about the lengths of the induction phase. These observations may imply a correlation between the rate of oxygen uptake found in the Schaal oven test and the acceleration in PV found by iodometric titration. These observations is supported by literature describing the propagation step to involve addition of oxygen to the alkyl radical ($R^{\cdot}$) forming a high energy peroxyl radical ($ROO^{\cdot}$). Because of its high energy the peroxyl radical react

rapidly to form a hydroperoxide by hydrogen abstraction and in this way causes the PV to increase (Damodaran et al., 2008).

From the PV measurement data presented in figure 3.2.2.2 it is also observed how the PV for the samples stored at 50 degrees suddenly decrease at approximately 150 hours of storage. A decrease in PV is also observed for the sample stored at 40 degrees, at approximately 200 hours of storage. These observations are suggested to be caused by decomposition of the primary oxidation products to secondary oxidation products. From literature it is known that as the lipid oxidation proceeds the concentration of hydroperoxides eventually starts to decrease as the rate of formation falls below the rate of decomposition (Frankel, 2005). That the decrease is observed in the sample stored at 50 degrees before it is observed in the sample stored at 40 degrees are suggested to be explained by the higher storage temperature of this sample, which cause the lipid oxidation reactions to proceed faster in this sample.

TBARS results

To monitor the development of secondary oxidation products during storage of the TRAN fish oil at the different storage temperatures, the TBARS assay was performed. The procedure was performed as described in section 2.5.5 and the results are presented in figure 3.2.2.3 and figure 3.2.2.4. Each of the curves represents a storage temperature and the TBARS measurements are given in µM/g oil as a function of time given in hours. The TBARS results for each sample are mainly given as the mean of three parallels. The uncertainty is given as the standard deviation and is shown in the figures as error bars. All data for the TBARS calculations are given in appendix F.

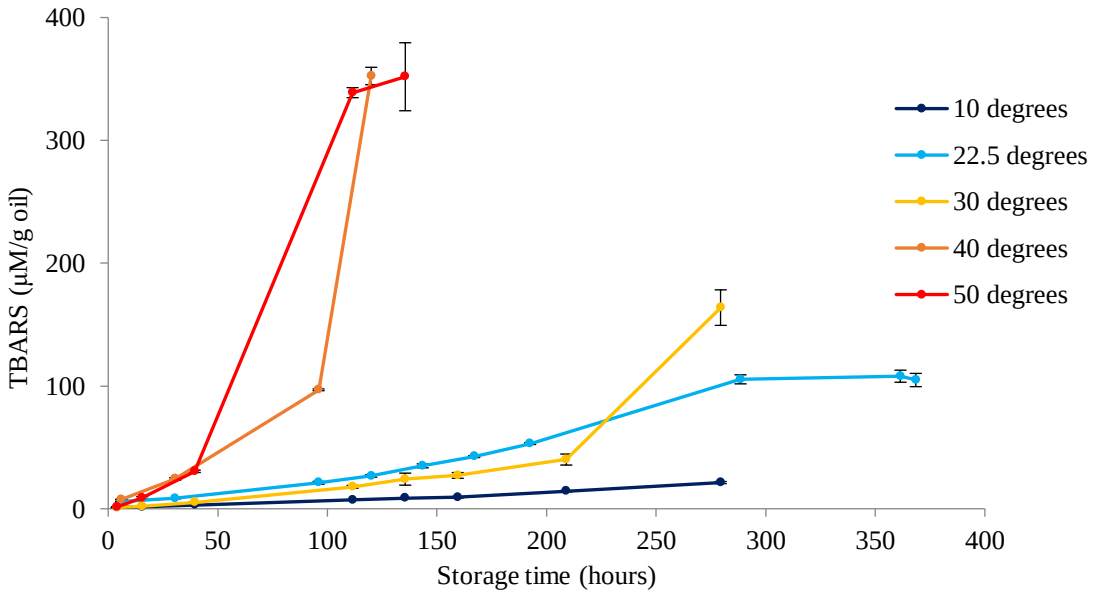

Figure 3.2.2.3: TBARS results for the TRAN oil samples stored at the five storage temperatures 10, 22.5, 30, 40 and 50 degrees. Each curve represents a storage temperature and the TBARS measurements are given as μM TBARS per gram oil as a function of storage time in hours. The error bars show the standard deviation of the measurements.

In figure 3.2.2.3 only the first TBARS measurements for storage temperatures 40 and 50 degrees are shown, this to be able to compare the curves of all storage temperatures in the same figure. The figure show that the rate of TBARS development is higher for the fish oils stored at 40 and 50 degrees. It is observed that the initial formation develops in almost the same manner. However, at approximately 40 hours of storage the rate of formation in the sample stored at 50 degrees increases. For the sample stored at 40 degrees the rate increase is observed later, at approximately 95 hours of storage. These results imply that the storage temperature influence the rate of TBARS development, and these findings are supported by literature showing similar results (Indergård, Tolstorebrov, Larsen, & Eikevik, 2014). When fish oils are stored at higher temperatures, the lipid oxidation reactions are accelerated and the primary oxidations products, the hydroperoxides, more rapidly decomposed into secondary oxidation products which results in the TBARS accumulating faster (Damodaran et al., 2008).

For the lower storage temperatures, 10, 22.5 and 30 degrees the same trends were observed. The only deviation from the trend is the TBARS values measured for the samples stored at 20 degrees was found to be higher compared to the samples stored at 30 degrees. This relation continues until approximately 230 hours of storage, as presented in figure 3.2.2.3. However, the measurements performed at the samples stored at 20 degrees was not done at the same day as the samples stored at 30 degrees, neither with the same batch of TBA solution. This may have given rise to variations in reaction conditions, and as described in literature the TBARS assay is sensitive to such variations (Barriuso et al., 2013). This considered, the deviation from the trend observed for the relation between samples stored at 20 degrees and samples stored at 30 degrees might be due to the variations in reaction conditions during performance of the procedure. The TBARS values for the two storage temperatures were evaluated using formula 2.7.3 given in section 2.7 and the results were not found to be significantly different ($p>0.05$). This strengthens the suspicion that the deviant results are due to variations in the performance of the TBARS procedure.

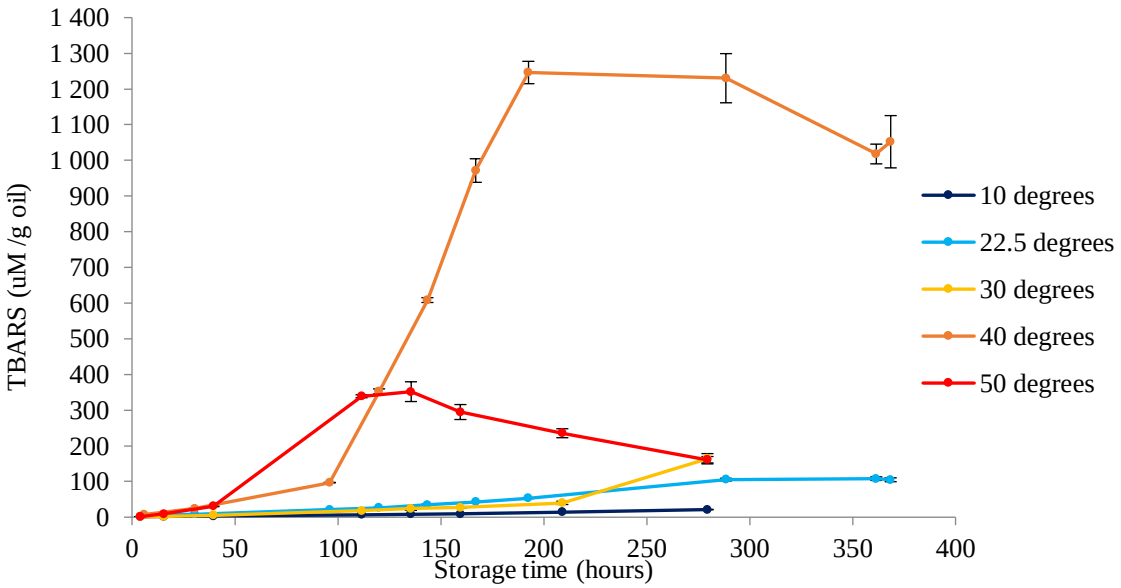

Figure 3.2.2.4: TBARS results for the TRAN oil samples stored at the five storage temperatures 10, 22.5, 30, 40 and 50 degrees. Each curve represents a storage temperature and the TBARS measurements are given as μM TBARS per gram oil as a function of storage time in hours. The error bars show the standard deviation of the measurements.

In figure 3.2.2.4 all TBARS measurements for all five storage temperatures are presented. As before each curve represents a storage temperature and the measured TBARS values are given in μM/g oil as a function of time given in hours. From figure 3.2.2.4 it is observed that after approximately 140 hours of storage the TBARS value for the sample stored at 40 degrees continuous to increase, while the TBARS value for the fish oil stored at 50 degrees cease and starts to decrease. This observation is suggested to be explained by the difference in storage temperature between the two samples. In the sample stored at 50 degrees the decomposition of TBARS into tertiary oxidation products may have progressed more rapidly compared to the sample stored at 40 degrees because of the higher storage temperature. The observed decrease for the samples stored at 50 degrees is therefore suggested to be caused by the rate of TBARS decomposition at this point, surpassing the rate of formation. This suggestion is supported by literature explaining how elevated temperatures increases the rate of lipid oxidation (Damodaran et al., 2008). For the fish oil stored at 40 degrees the decrease in TBARS value was observed later and suggests that storage at 40 degrees allows for higher accumulation of TBARS before the rate of decomposition passes the rate of formation. The difference in temperature may also influence on which secondary oxidation products are formed (Frankel, 2005). This may have led to differences regarding the quantity of TBA reactive substances present in the two fish oil samples.

3.3 The antioxidant experiment

In the antioxidant experiment the stabilizing effect of different antioxidant additives on different fish oils where tested by use of the Schaal oven test at the storage temperature of 50 degrees. The five fish oils used were TRAN, 18/12-S, 18/12-B, TG60 and TG90 and these were described in more detail in section 2.1. For each fish oil it was attempted to prepare seven different samples. One sample without antioxidant additive and six samples each with one type of antioxidant additive. The six antioxidant additives utilized was described in more detail in section 2.2. The antioxidants were added to the fish oil samples by the procedure described in section 2.5.2 and the blending was performed with accuracy to ensure equal initial conditions for all samples when entering the Schaal oven test. Some antioxidant additives did not dissolve homogeneously in all five fish oils and for that reason these samples were excluded from the experiment.

In the antioxidant experiments the reference zero weight used to calculate the weight increase data was set to the starting weight at 0 hours of storage for all sample except the sample containing antioxidant additive rosemary extract. The reason for choosing the weight at 0 hours of storage as the reference zero weight in these experiments was because the first weight increase measurement was performed at unequal times for the different fish oil samples. Fish oil samples containing the antioxidant additive rosemary extract was excepted from this rule because of the major weight decrease observed for these samples during the first two hours. The weight decrease in these samples was assumed to be caused by larger quantities of trace water and potentially other volatile compounds evaporating from the samples during the two first hours of storage. To avoid negative weight increase measurements for the samples added with rosemary extract, the reference zero weight used to calculate weight increase data for these samples was set to the weight measured after 2-5 hours depending on the fish oil. The exact reference zero weight used for each fish oil is emphasized in each of the following sections.

3.3.1 TRAN fish oil

In the TRAN fish oils all six antioxidant additives dissolved and the results from each sample, including the control without antioxidant additive (No AOX), are presented in figure 3.3.1.1. Each curve represents a specific sample and the measurements are presented as the weight increase given in g/100g oil as a function of storage time given in hours. All weight increase measurements are given as the mean of 3-4 parallels. The uncertainty of the measurements is given as standard deviation and is, together with all weight increase data, enclosed in appendix G.

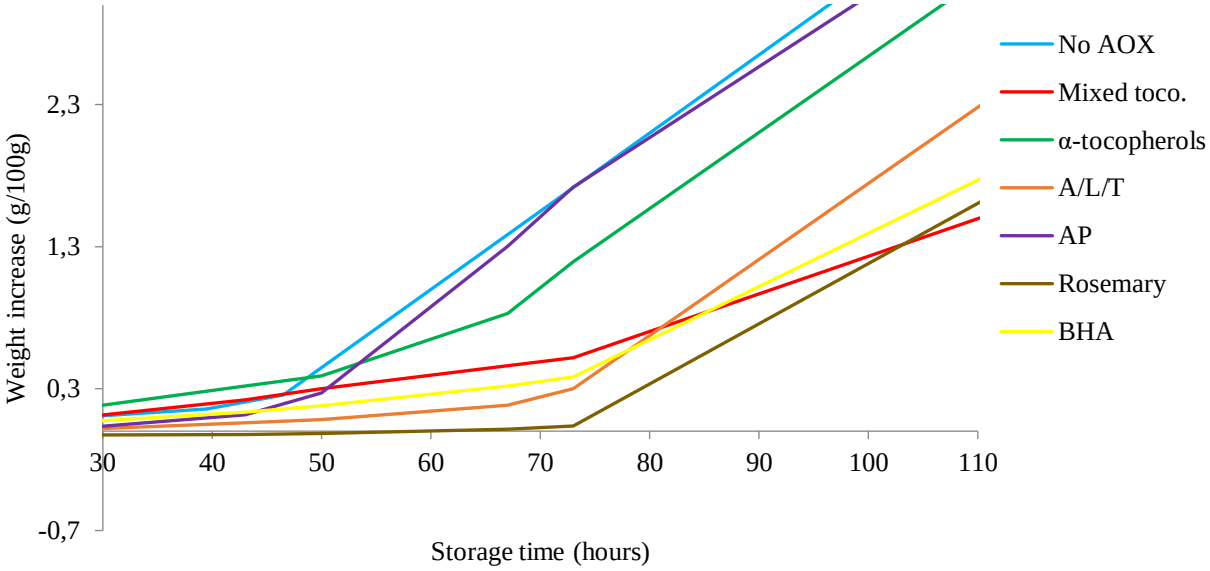

Figure 3.3.1.1: The result from the Schaal oven test performed for the TRAN fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive. The "No AOX" sample function as the control and are not added with an antioxidant additive. The weight increase results for each sample are given in g/100g oil as a function of storage time given in hours.

The results presented in figure 3.3.1.1 implies that all six antioxidant additives had a positive effect on the oxidative stability of the fish oil during storage at 50 degrees. This is observed by all curves reaching the end of the induction phase at a later stage compared to the control (No AOX). The end of the induction phase is identified, as described in section 1.4.4, as the point of maximum change in the weight increase rate. These results are also supported by previous research showing antioxidative activity for all antioxidative additives utilized in this experiment when added to fish oil samples (Fan & Eskin, 2015).

Even though all antioxidant additives are observed to positively affect the stability of the fish oil, some are shown to have significantly greater effect compared to others. In table 3.3.1.1 the approximate time for each sample to reach a weight increase of 0.5% are given to enable comparison between the different additives more easily.

Table 3.3.1.1: An overview of the approximate time in hours for each of the TRAN fish oil samples to reach a weight increase of 0.5%.

Sample code	Functioning antioxidants	Time to reach 0.5% weight increase (hours)
No AOX	None	51
AP	Ascorbyl palmitate	54
α-tocopherol	α-tocopherol	55
Mixed toco.	α-, β-, γ-, and δ-tocopherols	71
BHA	Butylated hydroxyanisole	76
A/L/T	Ascorbyl palmitate, lecithin and α-tocoperhol	77
Rosemary	Rosemary extract	83

From the results presented in figure 3.3.1.1 and table 3.3.1.1 the potency of the antioxidant additive was in the TRAN fish oil found to be in the following order.

rosemary > A/LT mixture > BHA > mixed toco. > α-tocopherol > AP

The antioxidant additive found to have the greatest effect on the oxidative stability of the TRAN fish oil was the rosemary extract which was found to delay the point of 0.5% weight increase by approximately 32 hours. The highly stabilizing effect observed by addition of rosemary extract to the cod liver oil is suggested to be caused by the fact that rosemary extracts is rich in phenolic compounds such as carnosic acid, carnosol and rosmarinic acid (Xie, VanAlstyne, Uhlir, & Yang, 2017). As described in section 1.3, the phenolic compounds are known to provide high antioxidant activity and function as radical scavengers, breaking the chain reactions of lipid oxidation through hydrogen atom donation or single electron transfer which results in radical neutralization. According to Mozuraityte et al. the antioxidants capable to perform radical scavenging are referred to as the most important class of antioxidants for protection of fish oils against lipid oxidation (Mozuraityte et al., 2016). This supports the stabilising effect observed by addition of rosemary extract to the TRAN fish oil in this experiment.

However, other phenolic antioxidants with radical scavenging properties, such as the tocopherols were also tested. The stronger effect observed for rosemary compared to the tocopherol containing additives can be explained by the polar paradox. The polar paradox is a theory explaining the controversial behaviour of antioxidants where polar antioxidants are more efficient in non-polar medias such as bulk fish oils and non-polar antioxidants in more polar medias such as oil-in-water emulsions. The theory was according to Shahidi and Zhong first described by Porter et al. in 1989 and is based on how the antioxidants physically orient in a given medium as a result of polarity (Shahidi & Zhong, 2011). While non-polar antioxidants are found oriented in the oil-water interface of emulsions, the polar antioxidants orients in the air-oil interface of non-polar media and thus provide higher protection in bulk fish oil substrates (Damodaran et al., 2008). The tocopherols are non-polar, or lipophilic, antioxidants which according to the polar paradox hence are less efficient in bulk fish oils compared to the antioxidant components of rosemary which consist of both polar and non-polar components (Xie et al., 2017). However, it should be emphasized that contradictions to the polar paradox have been described in recent research. In the re-evaluation by Shahidi and Zhong it is suggested that more complex factors beyond polarity must also be taken into account when explaining the efficiency of different antioxidants (Shahidi & Zhong, 2011).

The A/L/T mixture additive of the experiment, containing the antioxidants ascorbyl palmitate, lecithin and α-tocopherols was observed to have the second most stabilizing effect on the TRAN fish oil if based on how long the additives delayed the point of 0.5% weight increase. The additive was found to delay the point of 0.5% weight increase by approximately 26 hours compared to the control. An interesting observation was the significantly greater effect observed for the A/L/T additive, compared to the additive only containing α-tocopherols and the additive only containing ascorbyl palmitate (AP). All additives were added to the TRAN fish oil samples at the same concentrations (1000 ppm), still the A/LT mixture provided better protection. These observations suggest a synergistic relation between the components of the A/L/T mixture which are supported by previous research in which ascorbyl palmitate are observed to act synergistically with phenolic compounds, including the tocopherols (Gordon & Kourkimskå, 1995). The A/L/T mixture have also been found to show superior effect compared to a large array of other antioxidants in earlier studies on prevention of lipid oxidation in refined fish oils (Frankel & Meyer, 2000). The synergistic relation is attributed to the recycling of tocopherols by AP. Initially, each tocopherol molecule (TO) neutralize two peroxyl radicals. First the tocopherol molecule donates a hydrogen atom to a radical and by doing this is

converted to a tocopherol radical (TO˙). In the second reaction the tocopherol radical interacts with another free radical resulting in a non-radical termination product. However, in the presence of AP the effects of tocopherols are enhanced. Interaction of AP with the tocopherol radical results in recycling of the "consumed" tocopherol radical (TO˙) back to its initial state (TO), which permit the tocopherol molecules to each neutralize more than two lipid radicals (Buettner, 1993). This synergistic relation may result in prolonged antioxidative effects of the tocopherol and thus promote the oxidative stability of the fish oil, which might explain the stabilising effects observed for the A/L/T mixture in this experiment.

The lecithin component of the A/L/T mixture is also assumed to contribute to the total stabilising effect. In previous research the antioxidant activity of lecithin is attributed to the phospholipid components of lecithin which are suggested to have metal chelating properties. In literature this function is described as effective for the maintenance of oxidative stability in bulk fish oils (Judde, Villeneuve, Rossignol-Castera, & Le Guillou, 2003; Mozuraityte et al., 2016). However, in addition to the metal chelating properties, lecithin has also been described to function as an emulsifier in the A/L/T mixture by promoting the interaction between AP and the tocopherols and thus promoting the recycling processes of the tocopherols (Frankel & Meyer, 2000).

The observation that A/L/T mixture supplies more oxidative stability to the TRAN fish oil compared to the mixed tocopherol additive containing α-, β-, γ-, and δ-tocopherols based on the 0.5% weight increase mark, are suggested to be explained by the same synergistic reactions as discussed in the previous sections. However, from figure 3.3.1.1 it is observed that beyond the point of 0.5% weight increase, at approximately 80 hours of storage, the sample containing the A/L/T mixture starts to increase more rapidly in weight and surpasses the sample added with the mixed tocopherol additive. From the literature already cited this observation is suggested to be caused by a higher total concentration of tocopherols in the mixed tocopherol additive compared to the A/L/T mixture. Both additive was added to the fish oil samples in the same concentrations (1000 ppm), but since the A/L/T mixture contains α-tocopherol in combination with lecithin and ascorbyl palmitate, the overall tocopherol concentrations can reasonably be assumed to be lower in the A/L/T mixture compared to the mixed tocopherol additive. For that reason the change in weight increase rate observed for the A/L/T mixture at stages after the point of 0.5% weight increase is suggested to be caused by the A/L/T mixture at this point being depleted for tocopherols and ascorbyl palmitate to proceed the recycling process. However, the mixed tocopherol mixture assumed to contain a higher initial concentration of tocopherol is not

depleted and from this point on is therefore able to provide better protection compared to the A/L/T mixture. This is observed by a lower weight increase rate, reflecting a lower oxidation rate for the sample added with mixed tocopherols compared to the sample added with the A/L/T mixture.

The antioxidant containing only ascorbyl palmitate was in this experiment found to be the least efficient antioxidant in protection of the TRAN fish oil against the lipid oxidation reactions. The additive was found to delay the point of 0.5% weight increase by only approximately 3 hours compared to the control (No AOX), and from the figure 3.3.1 only a negligible effect is observed. Similar results were seen in research by Kasbo where ascorbyl palmitate also were found to distinguish as the least efficient antioxidant compared to other antioxidant additives tested when added in cod liver oil (Kasbo, 2012). The ascorbyl palmitate is an ester of ascorbic acid (vitamin C) and is known as the fat-soluble version of the ascorbic acid. In literature the ascorbyl palmitate is described to have both singlet oxygen quenching properties and trace metal chelating properties (Baştürk, Boran, & Javidipour, 2017; Lee et al., 1999). Gordon & Kourkimskå suggest that since ascorbyl palmitate performs its antioxidant activity by quenching oxygen from the substrate, the exposure to excess oxygen conditions, such as in this experiment, would have an inhibitory effect on the stabilisation provided by ascorbyl palmitate (Gordon & Kourkimskå, 1995). The ascorbyl palmitate is also as mentioned a lipophilic version of vitamin C and according to the polar paradox described earlier in this section this suggests that the ascorbyl palmitate is expected to perform better in oil-water emulsions rather than in bulk oil as tested in this experiment. This might also be a contributing factor regarding the poor antioxidant activity observed for this additive. It should also be mentioned that the ascorbyl palmitate in some occasions have been found to act prooxidative when added to fish oils in certain concentrations (Hamilton, Kalu, McNeill, Padley, & Pierce, 1998).

Previous research comparing the ascorbyl palmitate antioxidant efficiency against the efficiency of BHA in vegetable oils found that the ascorbyl palmitate provided better oxidative protection (Cort, 1974). This literature does not support the observations made in this experiment where BHA was found to perform significantly better than the ascorbyl palmitate additive despite that it was added in a lower concentrations (AP = 1000 ppm, BHA = 150 ppm). However, it is generally known that both the chemical composition of the oil, the concentration of the antioxidant additive, as well as the external conditions such as time, temperature and light conditions are factors which can influence the performance of antioxidants and this might explain the conflicting results. In addition, vegetable oils are naturally high in tocopherol

content which might explain the minor effects observed when suppling even more phenolic antioxidants to these oils (Cort, 1974). BHA is a phenolic compound which performs its antioxidant activity by scavenging radicals through hydrogen atom donations. The scavenging antioxidant activity is as already mentioned an important antioxidant function in protection of fish oils against lipid oxidation and this substantiate the results observed in this experiment compared to the results found in the experiments performed by Cort where vegetable oils were utilized (Cort, 1974).

The BHA additive was found to be less effective compared to rosemary extract. Considering both antioxidants are polar components, the polar paradox does not explain these observations. However, the observation may be explained by the lower concentrations utilized for BHA relative to the rosemary extract (BHA = 150 ppm, rosemary = 1000 ppm). Previous research by Gordon et al. support the results of this experiment to some extent, since rosemary also in this study was found to provide a greater antioxidant activity compared to BHA (Gordon & Kourkimskå, 1995). However, in this study rapeseed oil was utilized and the storage was performed at 100 degrees.

Another observation made from the results of this experiment is that the antioxidant containing only α-tocopherols show a significantly lower stabilizing effect on the TRAN fish oil compared to the additive containing a mixture of different tocopherols. The tocopherols are natural existing antioxidants, which more often goes by the name vitamin E. The different types of tocopherols are all derivates of 6-chromanol, but differ in the number and position of methyl groups attached to their phenolic ring (Kamal-Eldin & Budilarto, 2015). The potency of the different tocopherols as antioxidants are determined by the bond dissociation energy of the hydrogen atom at the hydroxyl group of the tocopherol, which further is affected by the position of the methyl groups attached to the phenolic ring. The results obtained from this experiment are not supported by previous research which suggest the antioxidant potency of tocopherols to be in the order of $\alpha > \beta > \gamma > \delta$ (Kamal-Eldin & Budilarto, 2015). For the results to be supported by this literature it would be expected for the additive only containing α-tocopherols to perform a greater stabilising effect, compared to the mixture of different tocopherols when added in the same concentrations (1000 ppm). This because the α-tocopherol is described as a stronger hydrogen donor than the other tocopherols as a result of the methyl groups orientation on the phenolic ring. However, it is mentioned in literature that the potency order $\alpha > \beta > \gamma > \delta$ are not always shown to be consistent, and that parameters such as concentrations of the tocopherols, and properties of the media they are added in may affect the potency order (Kamal-Eldin &

Budilarto, 2015). The deviant observations regarding the order of potency might imply a synergistic relation between the different tocopherols present in the mixed tocopherol additive which thus explains the greater performance of the mixed tocopherol additive compared to the additive containing only α-tocopherol. Another suggested explanation is that the additive only containing α-tocopherols, which have low bond association energy compared to the other tocopherols are more rapidly consumed. While the mixed tocopherol additive, being a weaker hydrogen donor ensures a more long-lasting protective effect. However, no literature supporting these suggestions were found.

3.3.2 The 18/12-S fish oil

In the 18/12-S fish oil all antioxidant additives, except the ascorbyl palmitate additive dissolved. Since the AP antioxidant additive did not dissolve in the sample it was excluded from the experiment. The Schaal oven results for the other samples, including the control without antioxidant additive (No AOX), are presented in figure 3.3.2.1. Each curve represents a specific sample and the measurements are presented as the weight increase given in g/100g oil as a function of storage time given in hours. The weight increase measurements for the sample added with rosemary extract was in this fish oil calculated with the reference zero weight being the weight measured after 4 hours of storage. All weight increase measurements are given as the mean of 3-4 parallels. The uncertainties of the measurements are given as standard deviation and is, together with all weight increase data, enclosed in appendix H.

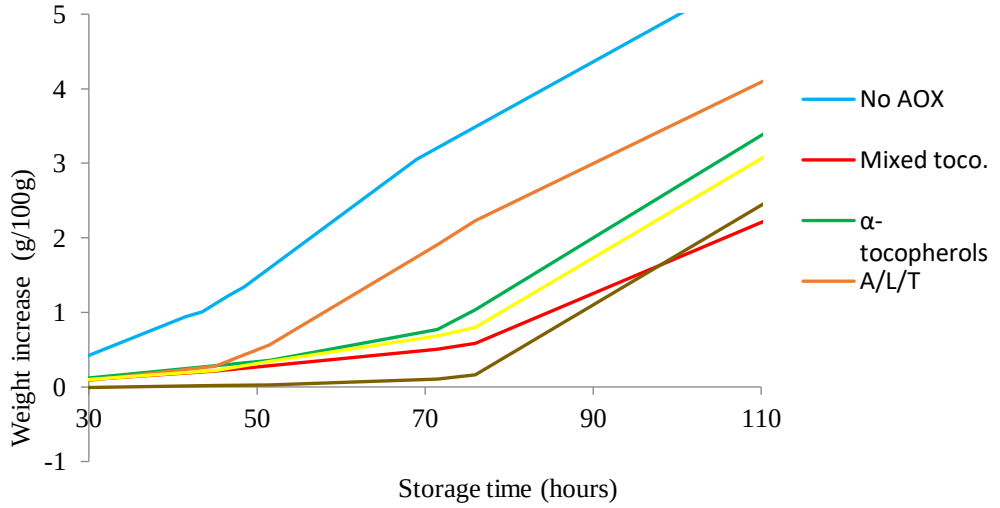

Figure 3.3.2.1: The result from the Schaal oven test performed for the 18/12-S fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive. The "No AOX" sample function as the control and are not added with an antioxidant additive. The weight increase results for each sample are given in g/100g oil as a function of storage time given in hours.

The results observed and presented in figure 3.3.2.1 implies that all five antioxidant additives applied a positive effect on the oxidative stability of the 18/12-S fish oil during storage at 50 degrees. This is observed by all curves reaching the end of the induction phase later than the control (No AOX). The end of the induction phase is identified, as described in section 1.4.4, as the point of maximum change in weight increase rate. These results are also supported by previous research showing antioxidative activity for all the antioxidative additives utilized in this experiment when added in fish oil samples (Fan & Eskin, 2015). In table 3.3.2.1 the approximate time for each sample to reach a weight increase of 0.5% are given to enable comparison between the different additives more easily.

Table 3.3.2.1: An overview of the approximate time in hours for each of the 18/12-s fish oil samples to reach a weight increase of 0.5%.

Sample code	Functioning antioxidants	Time to reach 0.5% weight increase (hours)
No AOX	None	32
A/L/T	Ascorbyl palmitate, lecithin, and α-tocopherol	49
α-tocopherols	α-tocopherol	58
BHA	BHA	60.5
Mixed toco.	Mixed tocopherol	71
Rosemary	Rosemary extract	81

From the results presented in figure 3.3.2.1 and table 3.3.2.1 the potency of the antioxidant additive in the 18/12-S fish oil was found to be in the following order.

Rosemary > mixed tocopherol > BHA > α-tocopherol > A/L/T mixture

These results are found to deviate from the results detected in the TRAN fish oil. However, rosemary extract is also in this fish oil found to be the most effective antioxidant and is observed to delay the time to reach a 0.5% weight increase by 49 hours compared to the control. This delay is greater than the delay observed in the TRAN fish oil (TRAN, rosemary delay = 32 hours). The sample containing rosemary is observed to reach the 0.5% weight increase at approximately 80 hours of storage in both oils, but the time for the two control samples to reach this point differ. In the TRAN fish oil the control was found to reach a 0.5% weight increase at approximately 51 hours of storage, as for the 18/12-S fish oil the 0.5% weight increase is observed at 32 hours of storage. These observations may be explained by the higher content of

PUFAs present in the 18/12-S fish oil compared to the TRAN fish oil (TRAN = 28.07%, 18/12-S = 36.44%) and is supported by the general knowledge of fish oils containing higher portions of PUFAs being more prone for lipid oxidation compared to the more saturated fish oils.

As before the great stabilising effects observed for the rosemary extract can be explained by the additive being rich in phenolic compounds known to provide high oxidative stability in fish oils by scavenging of free radicals. The mixed tocopherol additive was found to provide the second-best effect in the 18/12-S fish oil and was found to reach the 0.5% weight increase at 71 hours of storage, which is the same value as observed in the TRAN fish oil. As described in the previous section, the difference in effect observed between the mixed tocopherols and the rosemary extract, both containing radical scavenging phenolic compounds, may be explained by the polar paradox. As described in section 3.3.1, the theory of the polar paradox offers an explanation for why non-polar antioxidants such as the tocopherols provide less oxidative protection compared to more polar components such as those found in rosemary extracts.

The sample added with the α-tocopherol additive was also found to reach the point of 0.5% weight increase at the approximately same time as it did in the TRAN fish oil (TRAN = 55 hours, 18/12-S = 58 hours). As described in section 3.3.1 these results are not supported by previous research describing the α-tocopherol to be a greater hydrogen donor compared to the other tocopherols and for that reason the α-tocopherol additive would have been expected to provide a greater stabilising effect compared to the mixed tocopherol additive. However, this have shown to not always be consistent and the conflicting results are suggested to be caused by factors such as chemical composition of the fish oils, concentrations of additives and external conditions like time, temperature, and light conditions. In section 3.3.2 also some alternative explanations were discussed.

BHA is, as in the TRAN fish oil, found to provide an intermediate antioxidant activity compared to the other antioxidant additives. In the 18/12-S fish oil the time for the BHA sample to reach the 0.5% weight increase was found to be earlier compared to the TRAN fish oil (18/12-S =60.5 hours, TRAN = 76 hours), but compared to when the control of both fish oils reached the point of 0.5% weight increase, the delay performed by BHA was approximately the same in both oils (18/12-S = 28.5 hours, TRAN = 25 hours). It should also be pointed out, as already mentioned, that the concentration of BHA utilized was significantly lower compared to the other antioxidant additives (BHA = 150 ppm, others = 1000 ppm). Addition of BHA in higher concentrations may have resulted in increased antioxidant performance. Unfortunately, due to concerns regarding the toxicity effects of synthetic prepared antioxidants such as BHA the use

in edible oils are strictly regulated and concentrations in the range of which the other antioxidant additives was added are not permitted. As already mentioned in section 1.3.1 the maximum permitted levels of BHA in fish oils are set by the Council Directive No 95/2/EC to 200 mg/kg (200 ppm) (EFSA, 2011).

The additive containing only ascorbyl palmitate was as mentioned excluded from the experiment because of poor solubility in the 18/12-S fish oil. In literature it is described that despite the lipophilic properties of ascorbyl palmitate, which implies the antioxidant to have great solubility in oils, the ascorbyl palmitate is observed to require high temperatures in the range of approximately 100 degrees to properly dissolve (Kasbo, 2012). This may explain the poor solubility observed for ascorbyl palmitate when dissolution was attempted in room temperature conditions. These observations might also explain the poor antioxidant effects observed for the ascorbyl palmitate in the TRAN fish oil described in section 3.3.1. However, in the TRAN fish oil the ascorbyl palmitate did dissolve, but the stabilizing effects observed was almost negligible.

Considering the poor solubility observed for the ascorbyl palmitate additive, the decreased effect observed for the A/L/T mixture in the 18/12-S fish oil compared to the TRAN fish oil might also be explained by poor solubility of the ascorbyl palmitate. In the TRAN fish oil the A/L/T mixture was observed to delay the point of 0.5% weight increase by 26 hours compared to the control and was not observed to reach this point until 77 hours of storage. In the 18/12-S fish oil the delay of the 0.5% weight increase was observed to be 17 hours and the point was reach already at 49 hours of storage. These observations imply a decreased effect of the A/L/T mixture in the 18/12-S fish oil compared to the TRAN fish oil described in section 3.3.1. Considering that the additive only containing ascorbyl palmitate did not dissolve in the 18/12-S fish oil it might suggest that the A/L/T mixture neither was sufficiently dissolved, although it seemed to be. For that reason, the decreased antioxidative effect observed for the A/L/T mixture in the 18/12-S fish oil is suggested to be a result of the ascorbyl palmitate component not being sufficiently dissolved. Consequently, the ascorbyl palmitate would neither be able to enhance the antioxidant activity of the α-tocopherol component through synergistic interactions, as described in section 3.3.1, and thus result in the additive providing lower oxidative stability in the 18/12-S fish oil.

3.3.3 The 18/12-B fish oil

In the 18/12-B fish oil all antioxidant additives, except the additive containing ascorbyl palmitate, dissolved. Since the ascorbyl palmitate additive did not dissolve, this sample was excluded from the experiment. The Schaal oven results for the other samples, including the control without antioxidant additive (No AOX), are presented in figure 3.3.3.1. Each curve represents a specific sample and the measurements are presented as the weight increase given in g/100g oil as a function of storage time given in hours. The weight increase measurements for the sample added with rosemary extract was in this fish oil calculated with the reference zero weight being the weight measured after 5 hours of storage. All weight increase measurements are given as the mean of 3-4 parallels. The uncertainties of the measurements are given as standard deviation and is, together with all weight increase data, enclosed in appendix I.

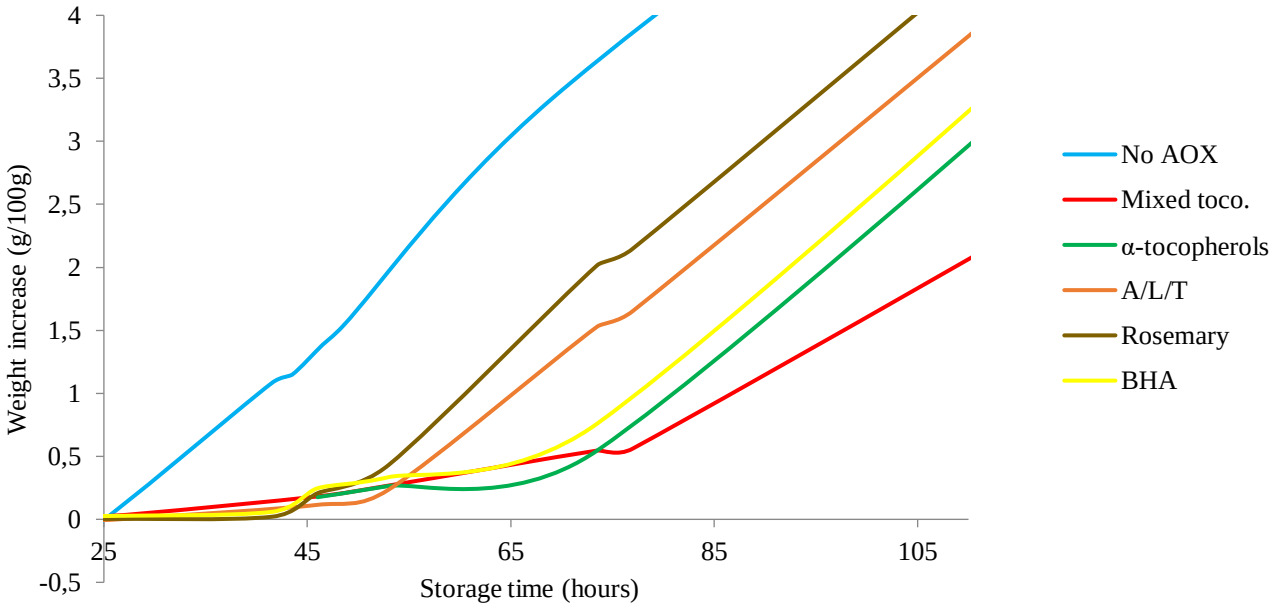

Figure 3.3.3.1: The result from the Schaal oven test performed for the 18/12-B fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive. The "No AOX" sample function as the control and are not added with an antioxidant additive. The weight increase results for each sample are given in g/100g oil as a function of storage time given in hours.

From the results presented in figure 3.3.3.1 all antioxidant additives were found to perform stabilising effect on the 18/12-B fish oil during storage at 50 degrees. As described in section 3.2.1 this was observed by all curves reaching the end of the induction phase at a later stage compared to the control (No AOX). These results are supported by previous research showing antioxidative activity for all the antioxidative additives utilized in this experiment when added

in fish oil (Fan & Eskin, 2015). The table 3.3.3.1 gives an overview of the approximate time for each sample to reach a weight increase of 0.5% and enable comparison between the different antioxidant additives more easily. The 18/12-B fish oil control was found to react the point of 0.5% weight increase after approximately the same time of storage as the 18/12-S fish oil control described in the previous section (18/12-B = 32.5 hours, 18/12-S = 32 hours).

Table 3.3.3.1: An overview of the approximate time in hours for each of the 18/12-B fish oil samples to reach an 0.5% weight increase.

Sample code	Functioning antioxidants	Time to reach 0.5% weight increase (hours)
No AOX	None	32.5
Rosemary	Rosemary extract	54
A/L/T	Ascorbyl palmitate, lecithin, and α-tocopherol	57.5
BHA	BHA	64
Mixed toco.	Mixed tocopherol	69
α-tocopherols	α-tocopherol	72

From the results presented in figure 3.3.3.1 and table 3.3.3.1 the potency of the antioxidant additive in the 18/12-B fish oil was found to be in the following order.

Mixed tocopherols > α-tocopherols > BHA > A/L/T mixture > rosemary

It should be noticed that even though the antioxidant additive containing only α-tocopherol in table 3.3.3.1 are listed as the additive to reach the point of 0.5% weight increase last, the figure 3.3.3.1 show that after this point the weight increase develops faster in the α-tocopherol sample compared to the mixed tocopherol sample and for that reason the mixed tocopherols are above listed as the most effective antioxidant additive in the 18/12-B fish oil.

The first observation made from these results is how the order of antioxidant potency varies compared to the potency order listed for the TRAN and the 18/12-S fish oils. The most apparent observation is how the rosemary extract, which was found to be the most effective antioxidant additive in both the TRAN and the 18/12-S fish oils, provides the least stabilising effect among the different antioxidant additives in the 18/12-B fish oil. In the 18/12-B fish oil the rosemary extract is found to delay the point of 0.5% weight increase by 21.5 hours and reaches this point already at 54 hours of storage. All external factors in relation to handling, such as the blending

procedure, antioxidant concentration, temperature, and light exposure, were kept constant for all fish oil samples. Considering this, the difference in antioxidant efficiency observed for rosemary in the 18/12-B fish oil compared to the 18/12-S and the TRAN fish oil is suggested to be due to differences in chemical composition of the fish oils. Similar results were published by Kasbo where rosemary extract was found to provide a greater antioxidative effect compared to mixed tocopherols when added to cod liver oil, but when added to fish oil concentrates the opposite was observed (Kasbo, 2012). The results from this study to some extent matches the variations observed between the TRAN and the 18/12-B fish oils of this experiment considering the difference in PUFA content of the two oils (TRAN = 28.07%, 18/12-B = 37.79%). However, it does not support the difference observed between the 18/12-B and the 18/12-S fish oils, considering the PUFA content of these two oils, in section 3.1, was found to not be significantly different.

As described in section 2.1 both the 18/12-S and the 18/12-B fish oils are anchovy fish oil R&D samples distributed by the same fish oil producer. However, the treatments which the oils have been receiving differ. While the 18/12-S fish oil have been treated by neutralisation and stripping. The 18/12-B fish oil have in addition been exposed to winterisation and bleaching. The difference in rosemary extract efficiency observed for the two fish oils might be a result of differences in the chemical composition of the oils as a result of different treatment protocols. Only the 18/12-B fish oil was for instance exposed to the bleaching step during refining. Bleaching is known to reduce the content of natural existing antioxidants. A change in the chemical composition such as removal of natural antioxidants might influence the performance of the added antioxidant additives and this could explain the different effects observed for rosemary extract in the 18/12-B and the 18/12-S fish oils. However, this is just a suggested explanation and no literature supporting the suggestions were found. The difference in time for which the rosemary samples of the 18/12-B and the 18/12-S fish oil were stored at 50 degrees before the reference zero weight was measured could have led to erroneous results. However, considering the negligible size of the difference (1 hour), it is reasonable to assume this not to be the case.

The additive containing the mixed tocopherols is observed, by the time to reach a 0.5% weight increase, to provide approximately the same effect in the 18/12-B fish oil as in the 18/12-S fish oil (18/12-S = 71 hours, 18/12-B = 69 hours). However, the α-tocopherol additive is observed to perform a better effect in the 18/12-B fish oil compared to the 18/12-S fish oil (18/12-S = 58 hours, 18/12-B = 72 hours). These observations may also be explained by differences in chemical composition as a result of the fish oils being exposed to different treatments during processing.

The BHA additive was also observed to provide a better effect in the 18/12-B fish oil compared to the 18/12-S fish oil when comparing the time to reach 0.5% weight increase (18/12-B = 64 hours, 18/12-S = 60.5). However, the difference in effect observed for BHA between the two fish oils was small compared to the difference in effect observed for the α-tocopherols. As already described in section 3.3.2 the intermediate effects of BHA is assumed to be caused by the lower concentrations utilized for BHA compared to the other phenolic antioxidant additives (BHA = 150 ppm, others = 1000 ppm).

The A/L/T mixture was found to provide a higher degree of oxidative protection in the 18/12-B fish oil compare to the 18/12-S fish oil when comparing the time for the samples to reach a 0.5% weight increase (18/12-B = 57 hours, 18/12-S = 49 hours). However, the potency of the A/L/T mix was still low compared to the other antioxidant additives, except for the rosemary extract as already mentioned that had the least effect in this fish oil. The difference observed between the 18/12-B and the 18/12-S fish oils is as before assumed to be caused by the difference in refining steps performed on the two oils. The difference in performance observed between the 18/12-B fish oil and the TRAN fish oil is, as explained in section 3.3.2 suggested to be caused by poor solubility of the A/L/T mixture in the 18/12-B fish oil. This suggestion is based on the observation that the additive only containing ascorbyl palmitate did not dissolve in the 18/12-B fish oil and was therefore excluded from the experiment.

3.3.4 The TG60 oil

In the TG60 fish oil only four out of six antioxidant additives dissolved. The additives which did not dissolve properly was the additive only containing ascorbyl palmitate and the additive containing the A/L/T mixture. For that reason, these samples were excluded from the experiment. The Schaal oven results for the remaining antioxidant additives, including the control without addition of antioxidants (No AOX), are presented in figure 3.3.4.1. Each curve represents a specific sample and the measurements are presented as the weight increase given in g/100g oil as a function of storage time given in hours. All weight increase measurements are given as the mean of 3-4 parallels. The uncertainties of the measurements are given as standard deviation and is, together with all weight increase data, enclosed in appendix J.

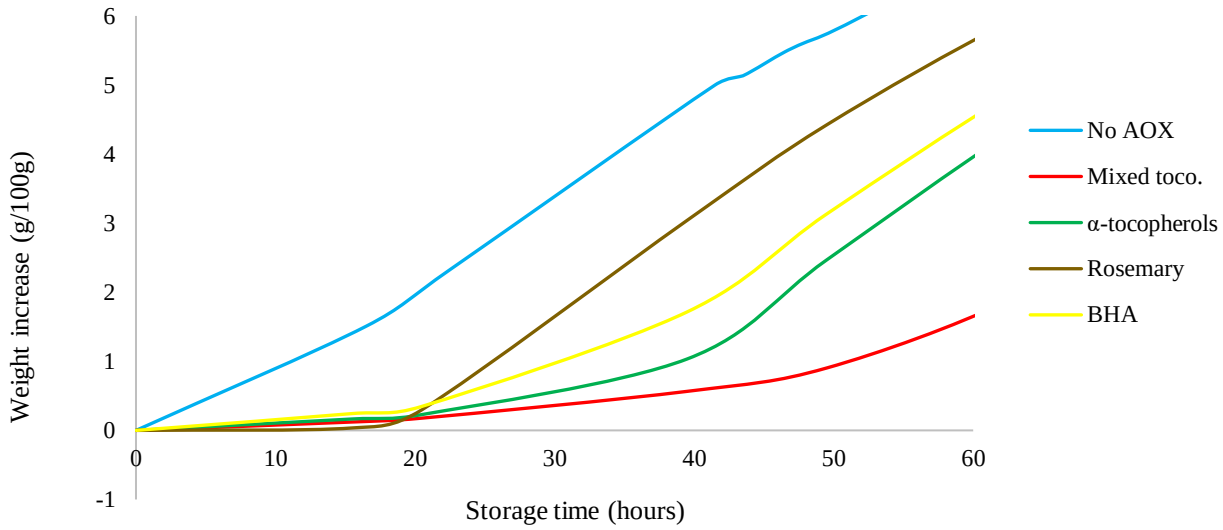

Figure 3.3.4.1: The result from the Schaal oven test performed for the TG60 fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive. The "No AOX" sample function as the control and are not added with an antioxidant additive. The weight increase results for each sample are given in g/100g oil as a function of storage time given in hours.

From the results presented in figure 3.3.4.1 all antioxidant additives were found to perform stabilising effect on the TG60 fish oil during storage at 50 degrees. As described in section 3.2.1 this was observed by all curves reaching the end of the induction phase after the control (No AOX). These results are supported by previous research showing antioxidative activity for all the antioxidative additives utilized in this experiment when added in fish oils (Fan & Eskin, 2015). The table 3.3.4.1 gives an overview of the approximate time for each sample to reach a weight increase of 0.5% and enable comparison between the different antioxidant additives more easily.

Table 3.3.4.1: An overview of the approximate time in hours for each of the TG60 fish oil samples to reach an 0.5% weight increase.

Sample code	Functioning antioxidants	Time to reach 0.5% weight increase (hours)
No AOX	None	5
Rosemary	Rosemary extract	22
BHA	BHA	23
α-tocopherol	α-tocopherol	28
Mixed toco.	Mixed tocopherols	37

From the results presented in figure 3.3.4.1 and table 3.3.4.1 the potency of the antioxidant additive in the TG60 fish oil was found to be in the following order.

Mixed tocopherols > α-tocopherols > BHA > rosemary

The potency order of the antioxidants of the TG60 oil was found to be equivalent to the order observed for the 18/12-B fish oil discussed in section 3.3.3. However, all additives were found to delay the point of 0.5% weight increase to a lower degree compared to in the 18/12-B fish oil. This observation is suggested to be explained by the difference in PUFA content of the fish oils (18/12-B = 37.79 %, TG60 = 71.66%). Considering the high degree of PUFAs present in the TG60 fish oil, the lipid oxidation reactions is expected to progress with a higher rate. This is also observed by the TG60 fish oil reaching the point of 0.5% weight increase already after 5 hours of storage at 50 degrees, while the 18/12-B fish oil reach this point at 32.5 hours of storage. The higher density of PUFAs present in the TG60 fish oil increases the probability of interactions between the PUFAs and the prooxidants present in the sample. This relation promotes the rate of the lipid oxidation in the fish oil and as the lipid oxidation propagates, this will result in a faster formation of lipid radicals compared to in the fish oil containing lower amounts of PUFAs. (Frankel, 2005). Higher concentrations of lipid radicals relative to the concentration of antioxidants, which were the same for both fish oils, explains the shorter delays observed for the antioxidant additives in the TG60 fish oil.

In the TG60 fish oil as for the 18/12-B fish oil the rosemary extract was observed to be the least efficient antioxidant additive. However, in the TRAN and the 18/12-S fish oils the rosemary was found to be the most efficient. These observations are already discussed in section 3.3.3 and was suggested to be due to variations in the fish oils chemical composition as a result of exposure to different treatment steps during refining. The treatments performed on the TG60 oil, as described in section 2.1, are neutralisation, stripping, transesterification to ethyl esters, concentration by distillation, transesterification back to TG, winterisation, and bleaching.

The most efficient antioxidant additive, when added to the TG60 fish oil, was found to be the mixed tocopherols. The mixed tocopherol additive was found to be provide significantly higher degree of oxidative stability compared to the other antioxidant additives. The great effects of the mixed tocopherol are assumed, as discussed in section 3.3.1, to be due to the phenolic structure of the tocopherols which make them perform well as radical scavengers through hydrogen donations. However, according to literature the observations of the mixed tocopherols to perform better than the additive only containing α-tocopherol are contradictory. However, this deviation was in section 3.3.1 suggested to be caused by factors such as in what media the antioxidants are added, in what concentrations and external factors like time, temperature and light conditions all which influence the antioxidant activity. A suggestion was also made about a possible synergistic relationship between the tocopherols of the mixed tocopherol additive, and that this would possibly explain the greater effects observed for the mixed tocopherol compared to the α-tocopherol additive. However, no literature supporting this suggestion was found.

The BHA additive was found to reach the point of 0.5% weight increase at approximately the same time as the rosemary extract (BHA = 23 hours, rosemary = 22 hours). However, as shown in figure 3.3.4.1 the effects of BHA is observed to be significantly greater compared to the rosemary extract beyond this point. As for all the other fish oil samples described in previous sections BHA is found to provide an intermediate protection compare to the other antioxidant additives. As already described in section 3.3.2 the intermediate effects of BHA is assumed to be caused by the lower concentrations utilized for BHA compared to the other antioxidant additives (BHA = 150 ppm, others = 1000 ppm).

In the TG60 oil neither the antioxidant additive containing the ascorbyl palmitate nor the one containing the A/L/T mixture dissolved. Problems with dissolving ascorbyl palmitate was also observed for the 18/12-S and the 18/12-B fish oils. The A/L/T mixture on the other hand was

observed to dissolve in both the 18/12-S and the 18/12-B fish oils, but a weaker antioxidant effect was observed if compared to the TRAN fish oil where both the A/L/T mixture and the ascorbyl palmitate additives dissolved. For that reason, the decreased effects observed for the A/L/T mixture in the 18/12-S and the 18/12-B fish oils was suggested to be due to lower solubility of the ascorbyl palmitate component of the A/L/T mixture in these oils compared to the TRAN fish oil. In literature, as described in section 3.3.2, the ascorbyl palmitate is, despite of lipophilic properties, observed to require high temperatures in the range of 100 degrees to properly dissolve (Kasbo, 2012). However, since differences in solubility is been observed for different fish oils, the requirement of higher temperatures seems to be associated with the chemical structure of the fish oils. Since neither the ascorbyl palmitate additive nor the A/L/T mixture was found to dissolve in the TG60 fish oil, this might imply a relation between the need for higher temperatures to dissolve ascorbyl palmitate and the degree of unsaturation. This suggestion is justified by the observed decrease in solubility for the ascorbyl palmitate with increased content of polyunsaturated fatty acids present. However, no literature supporting this observation was found.

3.3.5 The TG90 oil

In the TG90 fish oil only four out of six antioxidant additives dissolved. The additives which did not dissolve properly was also in this fish oil the additive only containing ascorbyl palmitate and the additive containing the A/L/T mixture. For that reason, these samples were excluded from the experiment. The Schaal oven results for the remaining antioxidant additives, including the control without addition of antioxidants (No AOX), are presented in figure 3.3.5.1. Each curve represents a specific sample and the measurements are presented as the weight increase given in g/100g oil as a function of storage time given in hours. All weight increase measurements are given as the mean of 3-4 parallels. The uncertainties of the measurements are given as standard deviation and is, together with all weight increase data, enclosed in appendix K.

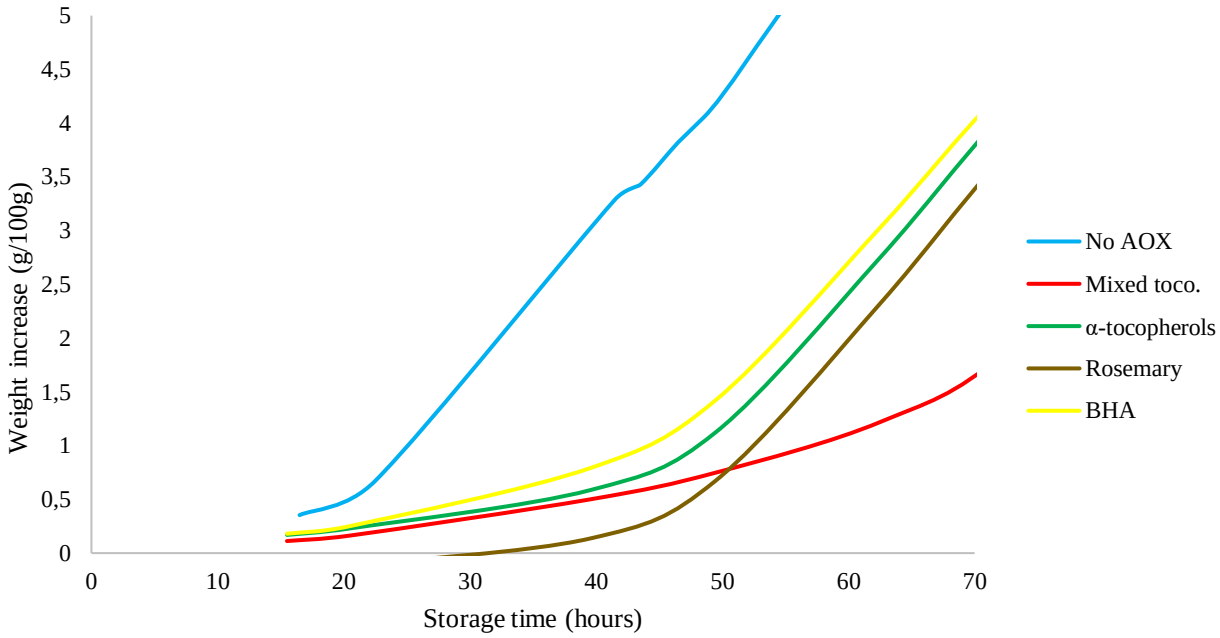

Figure 3.3.5.1: The result from the Schaal oven test performed for the TG90 fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive. The "No AOX" sample function as the control and are not added with an antioxidant additive. The weight increase results for each sample are given in g/100g oil as a function of storage time given in hours.

From the results presented in figure 3.3.5.1 all antioxidant additives were found to perform stabilising effect on the TG90 fish oil during storage at 50 degrees. As described in section 3.2.1 this was observed by all curves reaching the end of the induction phase after the control (No AOX). These results are supported by previous research showing antioxidative activity for all the antioxidative additives utilized in this experiment when added in fish oils (Fan & Eskin, 2015). The table 3.3.5.1 gives an overview of the approximate time for each sample to reach a weight increase of 0.5% and enable comparison between the different antioxidant additives more easily.

Table 3.3.5.1: An overview of the approximate time in hours for each of the TG90 fish oil samples to reach an 0.5% weight increase.

Sample code	Functioning antioxidants	Time to reach 0.5% weight increase (hours)
No AOX	None	20
F	BHA	30
A	α-tocopherol	35
B	Mixed tocopherols	39
E	Rosemary extract	47

From the results presented in figure 3.3.5.1 and table 3.3.5.1 the potency of the antioxidant additive in the TG90 fish oil was found to be in the following order.

Mixed tocopherols > rosemary > α-tocopherols > BHA

It should be noticed that even though the antioxidant additive containing rosemary extract was found to reach the point of 0.5% weight increase last, the figure 3.3.5 show that after this point the weight increase develops faster in the rosemary sample compared to the mixed tocopherol sample and for that reason the mixed tocopherols are above listed as the most effective antioxidant additive in the TG90 fish oil.

The mixed tocopherol antioxidant additive was observed to provide a more long-lasting effect compared to the other antioxidants in the TG90 fish oil. In initial stages of the storage period, up to approximately 50 hours of storage the rosemary extract was observed with significantly lower weight increase compared to the other antioxidant additives. However, from approximately 50 hours of storage, the rosemary extract, the α-tocopherols and the BHA

additives are observed to proceed their weight increase in an almost exponential manner. The weight increase of the mixed tocopherol sample on the other hand is observed to continue increasing more slowly.

The most deviating observation of the TG90 fish oil results compared to the results observed for the TG60 fish oil is the highly enhanced effects of the rosemary extract. While rosemary extract was found to perform the least stabilising effect in the TG60 fish oil and only delaying the point of 0.5% weight increase by 17 hours, the rosemary extract in the TG90 fish oil was observed to delay this point by approximately 27 hours and thus is found to perform greater effect compared to both the α-tocopherols and BHA. A reasonable explanation for the ambiguous results observed for rosemary extract in the different fish oils of this study is not found. However, suggestions regarding differences in chemical composition of the fish oils, which is known to influence the antioxidant activity, is proposed as a possible explanation (Damodaran et al., 2008). This was also discussed in section 3.3.3 and 3.3.4.

The observation of the mixed tocopherol additive to provide a higher degree of oxidative stability compared to the α-tocopherols have been shown to be persistent for all fish oils of this study. The contradictions of the observations in relation to literature stating a higher antioxidant potency for α-tocopherol compared to the other tocopherols (β, γ and δ) is discussed in all the previous sections 3.3.1-4. The deviant results have also been proposed to be a result of variations in factors shown to influence antioxidant activity such as antioxidant concentrations, chemical composition of the solvent, as well as external conditions such as time, temperature and light exposure. A suggestion was also made about a possible synergistic relationship between the tocopherols of the mixed tocopherol additive, and that this would possibly explain the greater effects observed for the mixed tocopherol compared to the α-tocopherol additive. However, no literature supporting this proposal was found.

BHA was found to be the additive of least stabilising effect in the TG90 fish oil. These results deviate from the results observed for the other fish oils of this study, where the BHA additive have shown an intermediate effect based on the potency order. However, the difference in time for the BHA sample to reach a weight increase of 0.5% (BHA = 30 hours) compared to the α-tocopherol containing additive in the TG90 fish oil is small (α-tocopherol = 35). From figure 3.3.5.1 it can also be observed that the development in weight increase for BHA is almost equal to the α-tocopherol additive throughout the experiment.

3.4 Comparison of result from Schaal oven test and OSI

A selection of samples tested in the Schaal oven test was also tested by determining of the OSI value. Not all samples from the Schaal oven test were tested because of time constraints, but the samples selected for the test were the TG60 fish oil samples and the TRAN fish oil samples. An overview of the samples tested is listed in table 3.4.1.

Table 3.4.1: An overview of the samples tested by determination of the OSI value

OSI Samples	
TRAN without antioxidants (control)	TG60 without antioxidants (control)
TRAN + Mixed tocopherols	TG60 + Mixed tocopherol
TRAN + α-tocopherols	TG60 + α-tocopherol
TRAN + A/L/T mixture	TG60 + Rosemary extract
TRAN + Ascorbyl palmitate	TG60 + BHA
TRAN + Rosemary extract	
TRAN + BHA	

The aim of measuring the OSI value for these samples was to compare the antioxidant potency order found during the Schaal oven test with the potency order found by measuring the OSI and to investigate for correlations. The storage temperature of both tests were 50 degrees and all fish oil samples containing antioxidant additives were taken from the same blend as utilized for the Schaal oven test to avoid variations related to differences in sample preparation. The concentration of antioxidant additive added to the fish oil samples was 1000 ppm, except for the BHA additive which were added in a concentration of 150 ppm.

3.4.1 The TRAN fish oil

In figure 3.4.1.1 the conductivity measurements for the TRAN fish oil samples are given as a function of time given in hours. Each curve represents a specific sample and the measurements are given as the mean of 3-4 parallels.

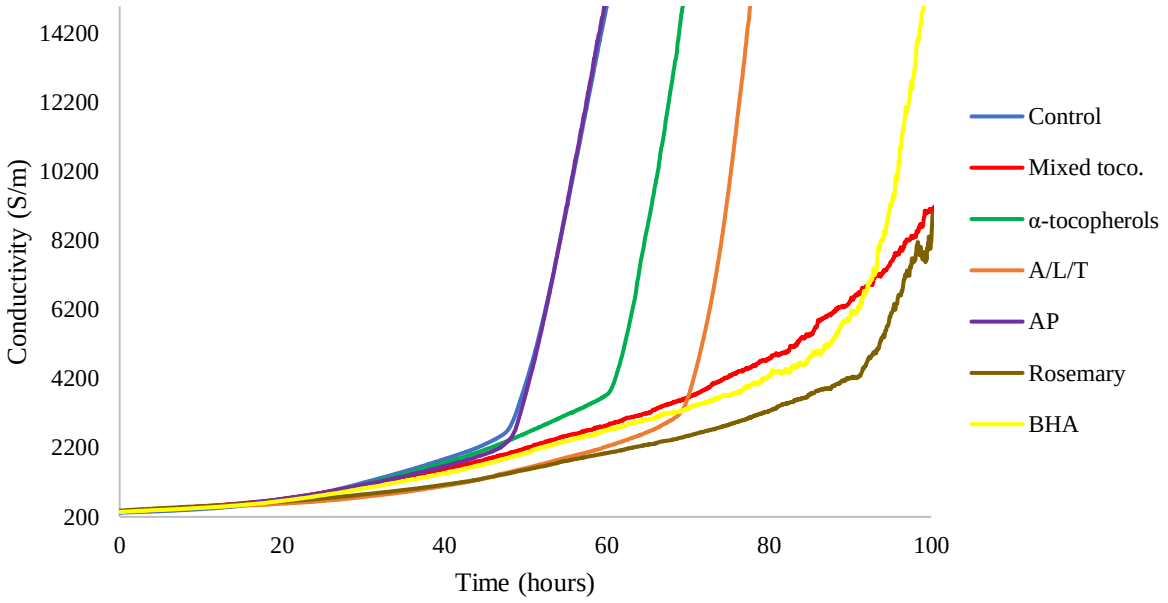

Figure 3.4.1.1: The result from the OSI performed for the TRAN fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive, except for the control which was not added with an antioxidant additive. The conductivity increase for each sample are given as a function of time given in hours.

In table 3.4.1.1 the times for maximum change in conductivity increase rate, the OSI value, are listed for all TRAN fish oil samples.

Table 3.4.1.1: An overview of the OSI values measured for the TRAN fish oil samples given in hours.

TRAN samples	OSI value (hours)	SD
Control	49.9	1.4
Ascorbyl palmitate	49.9	1.2
α-tocopherol	61.5	1.1
A/L/T mixture	70.9	2.0
Mixed tocopherols	91.8	9.2
Rosemary extract	95.4	3.9
BHA	97.3	3.1

From figure 3.4.1.1 and table 3.4.1.1 the potency order of the antioxidant additives added in TRAN fish oil was found to be as listed below.

rosemary > mixed toco. > BHA> A/L/T mixture > α-tocopherols > AP

Even though the BHA is listed as the last to reach the point of maximum change in conductivity increase rate, it is observed from figure 3.4.1.1 that the conductivity of BHA from this point, at approximately 97 hours, continuous to increase rapidly while the conductivity of the rosemary sample increases more slowly. This suggests a slower oxidation rate and a higher oxidative stability in the rosemary sample compared to the BHA even though the OSI of BHA occurred later. This is also true for the additive containing mixed tocopherols after the point of approximately 90 hours. Therefore, the samples added with these additives, are listed as more efficient in the antioxidant potency order above.

Compared to the potency order described for the same samples during the Schaal oven test described in section 3.3.1 some deviations are observed. In the Schaal oven test the order of antioxidant potency was found to be as listed below.

rosemary > A/L/T mixture > BHA > mixed toco. > α-tocopherol > AP

When comparing the potency orders of the two tests the deviation is found to apply for the additives in the middle section, the A/L/T mixture, BHA and the mixed tocopherols. However, evaluation of the times observed for the three middle section additives in the Schaal oven test show that the differences in time to reach a 0.5% weight increase was small (Mixed toco. = 71 hours, BHA = 76 hours, A/L/T = 77 hours) and for the A/L/T and BHA additives a significant difference was not found. The deviant results might also be caused by the antioxidant potency order of the Schaal oven test mainly being based on the time for the different additives to react a 0.5% weight increase, while the potency order observed from the OSI are based on the time for each oil sample to reach the point of maximum change in conductivity increase rate. For that reason, the plotted curves from each test was also compared and is presented in figure 3.4.1.2. From this comparison all the fish oil samples were observed to develop in the same trend in both the Schaal oven test and the OSI test.

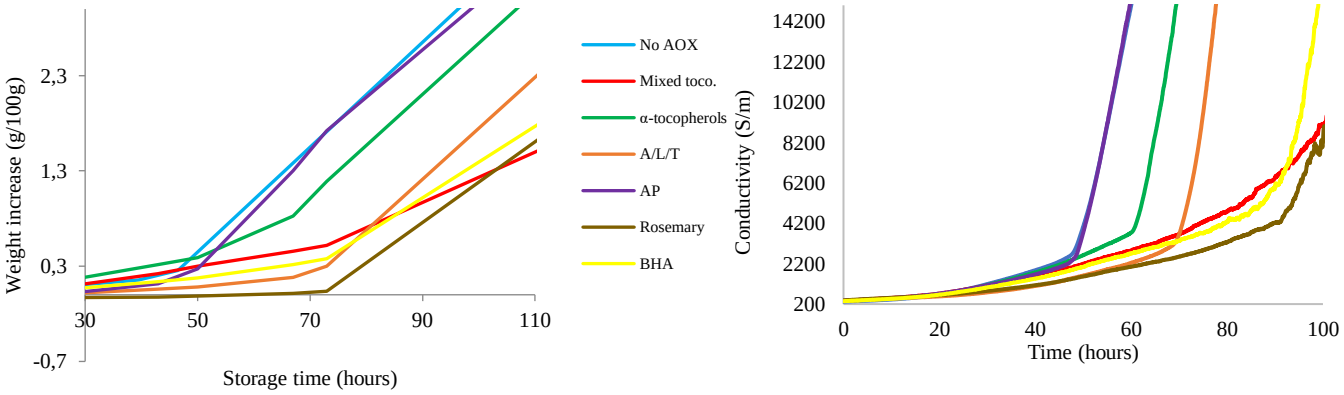

Figure 3.4.1.2: The plotted results from the two accelerated oxidation tests performed on the TRAN fish oil samples. On the left side the Schaal oven test results for the samples are plotted as weight increase in g/100g oil as a function of storage time given in hours. On the right side the OSI results for the samples are plotted as conductivity increase as a function of time given in hours.

In both test the rosemary additive was found to provide the highest level of oxidative stability, but both tests also show how the rosemary curve eventually crosses paths with the mixed tocopherol curve in the later stages of the tests. In early stages of the tests, the curves of the BHA and A/L/T mixture is in both tests observed to have results suggesting a lower oxidation rate compared to the mixed tocopherol sample. However, also observed for both tests, the oxidation eventually increases more rapidly in the BHA and A/L/T mixture samples which leads to these samples surpassing the mixed tocopherol sample which continues to propagate in a lower rate. Relative to the other samples, the α-tocopherol additive was also found to have the same development in both tests. The same goes for the AP samples which in both tests was found to reduce the lipid oxidation rate in only a negligible manner. Taking this comparison into account it can be suggested that the results from the two accelerated oxidation tests are found to correlate well.

3.4.2 The TG60 fish oil

In figure 3.4.2.1 the conductivity measures for the TG60 oil samples are given as a function of time given in hours. Each curve represents a specific sample and the measurements are given as the mean of 3-4 parallels.

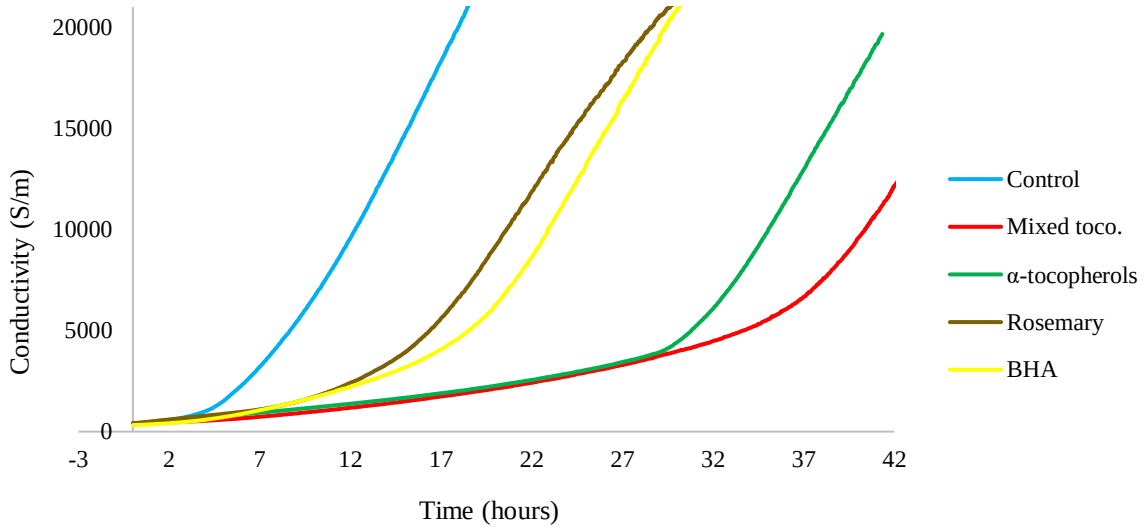

Figure 3.4.2.1: The result from the OSI performed for the TG60 fish oil samples with different antioxidant additives. Each curve represents a sample with a particular antioxidant additive, except the control sample which was not added with an antioxidant additive. The conductivity increase for each sample are given as a function of time given in hours.

In table 3.4.2.1 the time for the observed maximum change in rate of the increasing conductivity, the OSI value for the samples, are listed given in hours.

Table 3.4.2.1: An overview of the OSI values measured for the TRAN fish oil samples given in hours.

TRAN samples	OSI value (hours)	SD
Control	18.0	1.2
Rosemary extract	22.0	0.8
BHA	23.5	0.8
α-tocopherol	32.7	0.4
Mixed tocopherols	39.1	0.5

From the results presented in figure 3.4.2.1 and table 3.4.2.1 the potency order of the antioxidants was by the OSI test found to be as followed.

Mixed tocopherols > α-tocopherols > BHA > Rosemary

The order of antioxidant potency observed from the OSI test was found to match the potency order observed from the Schaal oven test. The only deviation observed is how the efficiency of the BHA additive in the Schaal oven test is found to be most similar to the efficiency observed for the α-tocopherol additive, while in the OSI test the efficiency of BHA is found to correlate more with the results observed for the rosemary extract. Unfortunately, no literature explaining this deviation was found. Except for this small deviation was the results from the two accelerated oxidation tests found to correlate well.

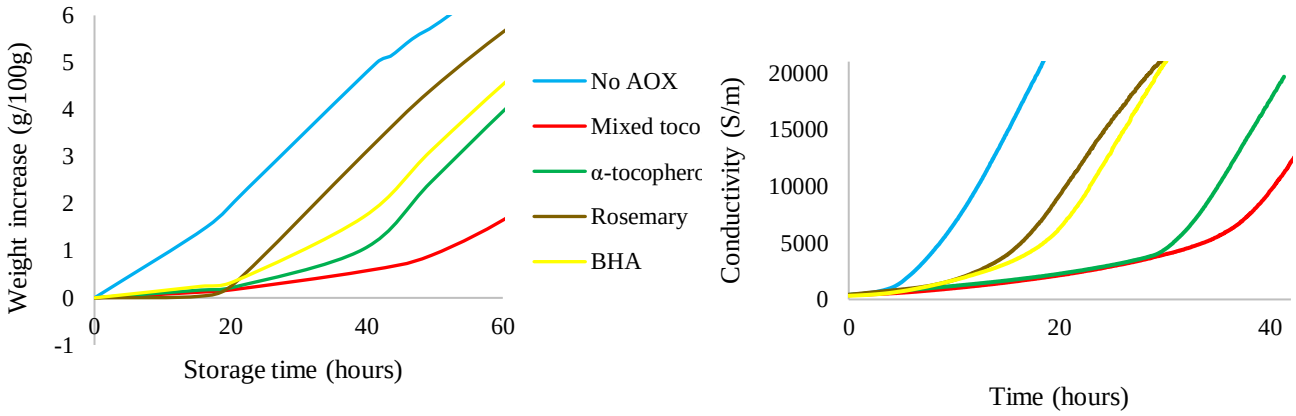

Figure: 3.4.2.2: The plotted results from the two accelerated oxidation tests performed on the TG60 fish oil samples. On the left side the Schaal oven test results for the samples are plotted as weight increase in g/100g oil as a function of storage time given in hours. On the right side the OSI results for the samples are plotted as conductivity increase as a function of time given in hours.

4. Conclusion

In this thesis lipid oxidation and the efficiency of different antioxidant additives in different fish oils were studied. Through Schaal oven tests performed on cod liver oil (TRAN) at different storage temperatures it was observed that the oxidative stability of the cod liver oil was reduced when exposed to increased storage temperatures. The results observed by measuring the PV and TBARS also showed how the oxidation products of the cod liver oil accumulates faster in the samples stored at higher temperatures. These observations are all supported by literature describing the accelerating effects of temperature on lipid oxidation reactions.

The Schaal oven test was also used to observe the antioxidant efficiency of six different antioxidant additives when added in different fish oils and stored at 50 degrees. The antioxidants utilized were rosemary extract, BHA, α-tocopherols, mixed tocopherols, A/L/T mixture and ascorbyl palmitate. The fish oils were a refined cod liver oil (TRAN), and four anchovy oils (12/18-S, 12/18-B, TG60, TG90) varying in the number of refining steps received. The antioxidant potency order established for each fish oil showed that rosemary extract was found to perform best in some of the fish oils, while in others the effect was poor. The efficiency of the α-tocopherol additive also varied between the fish oils, but it was always found to provide a lower oxidative stability compared to the mixed tocopherol additive. The mixed tocopherol additive was in general observed to provide good oxidative stability compared to the other samples and was in the anchovy oils with high PUFA content found to be the most efficient additive (TG60, TG90). The ascorbyl palmitate additive only dissolved in the cod liver oil where it provided poor, almost negligible, effect. The A/L/T mixture containing ascorbyl palmitate, lecithin and α-tocopherols was found to perform well in the initial stages of the oxidation when added in the cod liver oil. However, in the anchovy oils the efficiency was reduced and it did not dissolve in the anchovy fish oil concentrates (TG60, TG90). The BHA additive showed an intermediate activity compared to the other additives in all fish oil samples. However, these observations may be a result of the BHA being added in a lower concentration compared to the other six additives. The antioxidant efficiency measured in fish oils TG60 and TRAN was, in addition to the Schaal oven test, also measured by OSI. This was performed to evaluate for deviations between the two methods, but obvious deviations were not observed.

The variation in antioxidant effect observed for some of the antioxidant additives observed in this study emphasize the importance of performing antioxidant efficiency testing in the product of which the additive is attended. The antioxidant efficiency observed in one product does not necessarily correlate with the results observed in a similar product. It is also important to be aware that the results obtained under accelerated storage conditions, not necessarily correlate with the results obtained during storage at normal conditions. For that reason, the results of such tests should be critical evaluated before implementation.

5. Future work

A lot of different aspects revealed in this study would be interesting to investigate further. Regarding the performance of the antioxidant experiments it would be interesting to also investigate if the same antioxidant potency orders would be observed if the antioxidants were added in other concentrations, or if the experiments were performed at other storage temperatures. Considering the restrictions regarding the use of BHA as an antioxidant additive in food systems, it would also be interesting to further investigate for synergistic interaction between BHA and other antioxidant additives to possibly be able to enhance the antioxidant activity of BHA without the need for addition of higher concentrations. Regarding the poor effect observed for the AP containing additives it would have been interesting to investigate if the solubility would increase if addition were performed at higher temperatures and if this would lead to better results. Considering literature stating the low efficiency observed for AP also to be caused by the high oxygen access during storage, it would also be interesting to investigate for higher antioxidant efficiency by AP if the samples were exposed to less oxygen during storage. It would also be suggested to complete the OSI tests for all the antioxidant samples to further confirm the implied correlating relationship observed between the Schaal oven test results and the OSI in this study.

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Appendixes

Appendix A: AOCS recommended practice Cg 5-97

AOCS Recommended Practice Cg 5-97

Reapproved 1997

Oven Storage Test for Accelerated Aging of Oils

DEFINITION

This Recommended Practice describes the procedures for an accelerated test to measure the stability of oil samples by aging oils in an oven at 60°C. Methods such as peroxide value, sensory, gas chromatographic volatile compounds are then employed to measure oxidation levels and endpoints in the oils.

SCOPE

Applicable to edible fats and oils. This practice is based on optimum conditions that would be met by controlling the various parameters; any modifications will affect results.

PRINCIPLES

1. To create accelerated conditions for aging oils, storage temperature should be greater than ambient (approximately 25°C) but less than 80°C. High temperatures (generally above 80°C) are not representative of the same oxidation mechanisms that occur at 25°C (References, 1–2). In addition, ambient temperatures cause less reproducibility than higher temperatures because increased storage time permits more time for other environmental factors to affect the samples. Thus, temperatures within the 25–80°C range provide for accelerations in oxidation while retaining the same oxidation mechanism at lower temperature conditions but without the problems of long storage periods. A 60°C storage temperature is recommended based on the use of this temperature in an AOCS collaborative study of oil oxidation (3) and the use of this temperature in published studies of oil oxidation (4–6).
2. Oven storage tests should be conducted in the dark to avoid more than one oxidation mechanism such as both heat and light (References, 7–10).
3. Starting oils should have as little oxidation as possible to avoid having any oxidation products present in the oil acting as catalysts for further oxidation (References, 7–10). For example, an initial peroxide value of zero is recommended.
4. The surface to volume ratio[surface area (cm)/oil volume (mL)] is a critical factor affecting rate of oxidation (References 5,11). The ratio should remain constant from one trial to another.

GENERAL PRECAUTIONS

1. Glassware should be unetched and scrupulously clean to avoid any contamination such as oxidized material or trace metals. Detergents without surface-active agents should be used. Use deionized water for rinsing.
2. Do not expose oil to conditions that affect oxidation including high temperature, oxygen, metals, or light, prior to storage.

APPARATUS

1. Forced draft (mechanical convection) oven to operate at uniform sample temperature of 60°C ± 1°C. Refer to AOCS Specification H 1-39 for information about forced draft ovens.
2. Thermometer or thermocouple accurately calibrated.
3. Glass storage container; amber (or brown)-colored; clear glass may be used if precautions are taken to minimize exposure of oil to light while handling the samples before and after storage. Container size will be determined by amount of sample needed in tests.

PROCEDURES

1. Fill container with proper amount of oil, taking care to minimize exposure of oil to conditions such as heat, light, or metals that promote oxidation. Keep the surface to volume ratio [surface area (cm) /oil volume (mL)] (surface area = $r^2 \times 3.14$) constant from one sample to another. Examples of oil and storage containers include 250 mL in 8.8-cm beaker, 150 mL in 5.5-cm wide-mouth jar, and 75 mL in 4.5-cm beaker. Since the reaction mechanism is oxygen-dependent, as the ratio of volume to surface is increased, the oxidation rate will decrease. Narrow jars that are filled to a high level could have an altered oxidation reaction because of oxygen deficiency compared to the same container with less oil (12).
2. Oil storage may be either uncovered or covered. If storage containers are covered, keep top loosely closed or stoppered. Caps or tops should be lined with an inert material and it is recommended that corks and stoppers be wrapped in inert material such as cellophane or Teflon™ tape. When using open, uncovered containers, care should be taken to avoid cross-contamination of volatile compounds from the oil in one container to that of another container (References, 13).
3. If storage containers are covered, the amount of headspace in the containers should be uniform from one sample to another.
4. Separate storage containers are recommended for each sampling time rather than removing aliquots. Aliquots may be taken from the storage container if the amounts removed are very small and do not affect the surface to volume ratio.
5. After storage period ends, measure oxidation level immediately in the oil or sparge headspace of container with nitrogen, tightly stopper or cap container and store oil below 0°C until oxidation level can be measured.
6. To measure oxidation level or endpoint, use more than one method to determine extent of oxidation; prefer-

ably one each for primary and secondary oxidation products (References, 2). Tests to measure primary oxidation products include peroxide value (AOCS Cd8-53 or Cd 8b-90) and conjugated dienes (AOCS Ti 1a-64). Tests to measure secondary oxidation products include volatile organic compounds by gas chromatography (AOCS Cg 4-94), sensory evaluation (AOCS Cg 2-83), anisidine value (AOCS Cd 18-90), carbonyl value (JOCS 2.4.22-73). See References, 11–12 for reviews of methods to measure oxidation.

7. Take measurements over a period of storage time to produce an induction curve of the oxidation products. Initial and final rates of oxidation can also be calculated.

OPTIONS

1. The test may be modified to include gentle shaking of containers to increase rate of oxidation.

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Appendix B: AOCS Official Method Cd 8-53

Official 1960
Revised 1986
Reapproved 1989
Revised 1990, 1992, 1993

Peroxide Value
Acetic Acid–Chloroform Method

Definition: This method determines all substances, in terms of milliequivalents of peroxide per 1000 grams of sample, that oxidize potassium iodide (KI) under the conditions of the test. The substances are generally assumed to be peroxides or other similar products of fat oxidation.

Scope: Applicable to all normal fats and oils, including margarine. This method is highly empirical, and any variation in the test procedure may result in variation of results.

Apparatus

1. Pipet—0.5 mL, or other suitable volumetric apparatus capable of dispensing 0.5 mL of saturated potassium iodide (KI) solution.
2. Erlenmeyer flasks—with glass stoppers, 250 mL.

Reagents

1. Acetic acid–chloroform solution (3:2, v/v)—prepared by mixing 3 volumes of reagent-grade glacial acetic acid with 2 volumes of reagent-grade chloroform (see Notes, 1 and Caution).
2. Potassium iodide (KI) solution—saturated, prepared fresh each day analysis is performed by dissolving an excess of KI in recently boiled distilled water. Make certain the solution remains saturated during use, as indicated by the presence of undissolved KI crystals. Store in the dark when not in use. Test the saturated KI solution by adding 2 drops of starch solution to 0.5 mL of the KI solution in 30 mL of the acetic acid–chloroform solution. If a blue color is formed that requires more than 1 drop of 0.1 N sodium thiosulfate solution to discharge, discard the KI solution and prepare a fresh solution.
3. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) solution—0.1 N, accurately standardized vs. potassium dichromate primary standard as follows:
   (a) Sodium thiosulfate solution 0.1 N, prepared by dissolving 24.9 g of sodium thiosulfate in distilled water and diluting to 1 L.
   (b) The potassium dichromate primary standard should be finely ground, dried at 105 C for 2 hr and cooled in a desiccator. Weigh 0.16–0.22 g of potassium dichromate into a 500-mL flask or bottle by difference from a weighing bottle. Dissolve in 25 mL of water, add 5 mL of concentrated hydrochloric acid, 20 mL of potassium iodine solution (Reagents, 2) and rotate to mix. Allow to stand for 5 min and then add 100 mL of distilled water. Titrate with sodium thiosulfate solution, shaking continuously until yellow color has *almost* disappeared. Add 1–2 mL of starch indicator and continue the titration, adding the thiosulfate solution slowly until the blue color just disappears. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

$$\text{Normality of } Na_2S_2O_3 \text{ solution} = \frac{20.394 \text{ wt of } K_2Cr_2O_7}{\text{mL of sodium thiosulfate}}$$

4. Sodium thiosulfate solution, 0.01 N—accurately standardized. This solution may be prepared by accurately pipetting 100 mL of 0.1 N sodium thiosulfate into a 1000-mL volumetric flask and accurately diluting to volume with recently boiled distilled water.
5. Starch indicator solution—tested for sensitivity, prepared by making a paste with 1 g of starch (see Notes, 2) and a small amount of cold distilled water. Add, while stirring, to 200 mL of boiling water and boil for a few seconds. Immediately remove from heat and cool. Salicylic acid (1.25 g/L) may be added to preserve the indicator. If long storage is required, the solution must be kept in a refrigerator at 4–10 C. Fresh indicator must be prepared when the end point of the titration from blue to colorless fails to be sharp. If stored under refrigeration, the starch solution should be stable for about 2–3 weeks.

   Test for sensitivity—Place 5 mL of starch solution in 100 mL of water and add 0.05 mL of *freshly prepared* 0.1 N KI solution and one drop of a 50 ppm chlorine solution made by diluting 1 mL of a commercial 5% sodium hypochlorite (NaOCl) solution to 1000 mL. The deep blue color produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate.

Procedure for Fats and Oils

1. Weigh 5.00 ± 0.05 g of sample into a 250-mL Erlenmeyer flask with glass stopper and add 30 mL of the 3:2 acetic acid–chloroform solution. Swirl to dissolve the sample. Add 0.5 mL of saturated KI solution using a suitable volumetric pipet.
2. Allow the solution to stand with occasional shaking for *exactly* 1 min, and then immediately add 30 mL of distilled water (see Notes, 3 and References, 1).
3. Titrate with 0.1 N sodium thiosulfate, adding it gradually and with constant agitation. Continue the titration until the yellow iodine color has *almost* disappeared. Add about 0.5 mL of starch indicator solution. Continue the titration with constant agitation, especially near the end point, to liberate all of the iodine from the solvent layer. Add the thiosulfate solution dropwise until the blue color just disappears (see Notes, 4).

4. Conduct a blank determination of the reagents daily. The blank titration must not exceed 0.1 mL of the 0.1 N sodium thiosulfate solution.

Procedure for Margarine

1. Melt the sample by heating with constant stirring on a hot plate set at low heat, or by heating in an air oven at 60–70 C. Avoid excess heating and particularly prolonged exposure of the oil to temperatures above 40 C.
2. When completely melted, remove the sample from the hot plate or oven and allow to settle in a warm place until the aqueous portion and most of the milk solids have settled to the bottom.
3. Decant the oil into a clean beaker and filter through a Whatman no. 4 paper (or equivalent) into another clean beaker. Do not reheat for filtration unless absolutely necessary. The sample must be clear and brilliant.
4. Proceed as directed in Procedure for Fats and Oils, paragraphs 1–4.

Calculations

1. Peroxide value (milliequivalents peroxide/1000 g sample) =

$$\frac{(S - B) \times N \times 1000}{\text{wt of sample, g}}$$

Where—

B = titration of blank, mL
S = titration of sample, mL
N = normality of sodium thiosulfate solution

Notes

Caution

Chloroform is a known carcinogen. It is toxic by inhalation and has anesthetic properties. Avoid contact with the skin. Prolonged inhalation or ingestion can lead to liver and kidney damage and may be fatal. It is nonflammable, but will burn on prolonged exposure to flame or high temperature. The TLV is 10 ppm in air. A fume hood should be used at all times when using chloroform.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Numbered Notes

1. Isooctane has been proposed as a replacement for chloroform in this method. The method using isooctane was approved by the AOCS Uniform Methods Committee in 1990 as AOCS Official Method Cd 8b-90 and first appeared in the second printing of the 4th Edition of *Official Methods and Recommended Practices of the American Oil Chemists' Society.* The isooctane method is preferred due to the elimination of chloroform. It is the intention of the AOCS Uniform Methods Committee to delete the acetic acid–chloroform version of the method (AOCS Official Method Cd 8-53) from *Official Methods* within the next several years.
2. "Potato Starch for Iodometry" is recommended, because this starch produces a deep blue color in the presence of the iodonium ion. "Soluble Starch" is not recommended because a consistent deep blue color may not be developed when some soluble starches interact with the iodonium ion. The following are suitable starches: Soluble Starch for Iodometry, Fisher S516-100; Soluble Potato Starch, Sigma S-2630; Soluble Potato Starch for Iodometry, J. T. Baker 4006-04.
3. The test should be carried out in diffuse daylight or in artificial light shielded from a direct light source. A report on a coulometric method for the measurement of peroxide value (References, 2) indicates that the iodide–peroxide reaction is complete at the end of 1 min, and that the liberation of iodine is affected by light.
4. If the titration is less than 0.5 mL using 0.1 N sodium thiosulfate, repeat the determination using 0.01 N sodium thiosulfate.

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Appendix C: Experimental data from determination of fatty acid composition by GC-FID analysis.

Table C1: An overview of the results from determination of fatty acid composition in the TRAN fish oil by GC-FID analysis.

TRAN Fatty acid	I	II	MEAN	SD
C14:0	4.94	4.92	4.93	0.02
C14:1	0.46	0.46	0.46	0.00
C15:0	0.40	0.40	0.40	0.00
C16:0	11.46	11.41	11.43	0.03
C16:1	11.30	11.31	11.30	0.01
C17:0	0.55	0.55	0.55	0.00
C17:1	0.30	0.31	0.30	0.00
C18:0	2.30	2.31	2.31	0.01
C18:1n11+n9	17.94	17.88	17.91	0.04
C18:1n7	5.17	5.14	5.15	0.02
C18:2n6	2.24	2.22	2.23	0.01
C18:3n6	0.25	0.25	0.25	0.00
C18:3n3	0.98	0.98	0.98	0.00
c18:4n3	2.97	2.97	2.97	0.00
C20:0	0.07	0.07	0.07	0.00
C20:1	11.99	12.00	11.99	0.01
C20:2n6	0.30	0.31	0.30	0.01
c20:3n6	0.08	0.08	0.08	0.00
C20:4n6	0.06	0.06	0.06	0.00
C20:3n3	0.12	0.12	0.12	0.00
c20:4n3	0.63	0.63	0.63	0.00
C20:5n3	8.83	8.77	8.80	0.04
C22:0	0.08	0.08	0.08	0.00
c22:1n11	4.28	4.28	4.28	0.00
C22:1n9	0.51	0.51	0.51	0.00
C22:2	0.37	0.38	0.37	0.00
C22:3	0.04	0.04	0.04	0.00
C22:4	0.12	0.12	0.12	0.00
c22:5n3	0.96	0.96	0.96	0.00
C24:0	0.00	0.00	0.00	0.00
C22:6n3	10.06	10.24	10.15	0.12
C24:1n9	0.24	0.24	0.24	0.00
Sum	100.00	100.00		

Saturated	19.80	19.75	19.77	0.03
Monosaturated	52.19	52.12	52.16	0.05
Polysaturated	28.02	28.13	28.07	0.08
Omega-3	24.55	24.67	24.61	0.08

Table C2: An overview of the results from determination of fatty acid composition in the 18/12-S fish oil by GC-FID analysis.

18/12-S Fatty acid	I	II	MEAN	SD
C14:0	9.40	9.35	9.37	0.03
C14:1	0.36	0.32	0.34	0.02
C15:0	0.67	0.61	0.64	0.04
C16:0	21.37	21.30	21.33	0.05
C16:1	10.07	10.02	10.05	0.03
C17:0	1.21	1.19	1.20	0.02
C17:1	1.68	1.62	1.65	0.04
C18:0	4.13	3.96	4.04	0.12
C18:1n11+n9	10.55	10.44	10.50	0.07
C18:1n7	1.24	3.12	2.18	1.33
C18:2n6	1.23	1.13	1.18	0.08
C18:3n6	0.34	0.31	0.33	0.02
C18:3n3	0.90	0.82	0.86	0.05
c18:4n3	3.49	3.35	3.42	0.09
C20:0	0.26	0.24	0.25	0.01
C20:1	1.26	1.17	1.22	0.06
C20:2n6	0.17	0.16	0.17	0.00
c20:3n6	0.17	0.16	0.17	0.01
C20:4n6	0.52	0.44	0.48	0.06
C20:3n3	0.15	0.15	0.15	0.00
c20:4n3	0.76	0.70	0.73	0.04
C20:5n3	16.32	15.79	16.06	0.37
C22:0	0.15	0.14	0.14	0.01
c22:1n11	0.28	0.26	0.27	0.01
C22:1n9	0.13	0.12	0.13	0.00
C22:2	0.67	0.62	0.64	0.04
C22:3	0.07	0.07	0.07	0.00
C22:4	0.26	0.25	0.26	0.01
c22:5n3	1.51	1.47	1.49	0.02
C24:0	0.00	0.00	0.00	0.00
C22:6n3	10.43	10.46	10.44	0.02
C24:1n9	0.27	0.24	0.26	0.02
Sum	100.00	100.00		

Saturated	37.18	36.79	36.99	0.28
Monosaturated	25.82	27.33	26.57	1.06
Polysaturated	36.99	35.89	36.44	0.78
Omega-3	33.56	32.74	33.15	0.57

Table C3: An overview of the results from determination of fatty acid composition in the 18/12-B fish oil by GC-FID analysis.

18/12-B Fatty acid	I	II	MEAN	SD
C14:0	9.24	9.22	9.23	0.02
C14:1	0.28	0.28	0.28	0.00
C15:0	0.60	0.60	0.60	0.00
C16:0	18.93	18.84	18.88	0.06
C16:1	11.27	11.24	11.25	0.02
C17:0	1.38	1.39	1.39	0.00
C17:1	1.75	1.75	1.75	0.00
C18:0	3.45	3.43	3.44	0.02
C18:1n11+n9	9.72	9.67	9.69	0.03
C18:1n7	3.36	3.37	3.37	0.00
C18:2n6	1.26	1,26	1.26	0.00
C18:3n6	0.30	0,30	0.30	0.00
C18:3n3	0.78	0.79	0.79	0.00
c18:4n3	3.20	3.21	3.20	0.01
C20:0	0.21	0.20	0.21	0.01
C20:1	1.20	1.18	1.19	0.02
C20:2n6	0.17	0.17	0.17	0.00
c20:3n6	0.20	0.19	0.20	0.01
C20:4n6	0.05	0.05	0.05	0.00
C20:3n3	0.12	0.11	0.11	0.01
c20:4n3	0.75	0.75	0.75	0.00
C20:5n3	18.34	18.47	18.41	0.09
C22:0	0.16	0.15	0.15	0.01
c22:1n11	0.39	0.38	0.39	0.00
C22:1n9	0.14	0.13	0.14	0.01
C22:2	0.69	0.70	0.69	0.00
C22:3	0.07	0.07	0.07	0.00
C22:4	0.27	0.27	0.27	0.00
c22:5n3	1.73	1.74	1.73	0.01
C24:0	0.00	0.00	0.00	0.00
C22:6n3	9.70	9.86	9.78	0.11
C24:1n9	0.28	0.26	0.27	0.02
Sum	100.00	100.00		

Saturated	33.98	33.81	33.90	0.12
Monosaturated	28.38	28.25	28.32	0.09
Polysaturated	37.64	37.94	37.79	0.21
Omega-3	34.62	34.93	34.78	0.21

Table C4: An overview of the results from determination of fatty acid composition in the TG60 fish oil by GC-FID analysis.

TG60 Fatty acid	I	II	MEAN	SD
C14:0	0.13	0.13	0.13	0.00
C14:1	0.02	0.01	0.01	0.00
C15:0	0.02	0.02	0.02	0.00
C16:0	2.76	2.79	2.77	0.02
C16:1	0.88	0.88	0.88	0.00
C17:0	0.13	0.13	0.13	0.00
C17:1	0.13	0.12	0.12	0.01
C18:0	5.28	5.29	5.28	0.01
C18:1n11+n9	11.03	11.09	11.06	0.04
C18:1n7	3.45	3.45	3.45	0.00
C18:2n6	1.18	1.18	1.18	0.01
C18:3n6	0.26	0.26	0.26	0.00
C18:3n3	0.91	0.93	0.92	0.01
c18:4n3	3.13	3.16	3.14	0.02
C20:0	0.53	0.52	0.52	0.01
C20:1	2.76	2.75	2.76	0.01
C20:2n6	0.39	0.39	0.39	0.00
c20:3n6	0.38	0.38	0.38	0.00
C20:4n6	0.08	0.09	0.09	0.00
C20:3n3	0.29	0.29	0.29	0.00
c20:4n3	1.68	1.69	1.69	0.01
C20:5n3	36.05	36.12	36.08	0.05
C22:0	0.24	0.23	0.23	0.01
c22:1n11	0.50	0.50	0.50	0.00
C22:1n9	0.23	0.22	0.22	0.01
C22:2	1.48	1.48	1.48	0.00
C22:3	0.14	0.14	0.14	0.00
C22:4	0.55	0.55	0.55	0.00
c22:5n3	3.02	3.00	3.01	0.01
C24:0	0.00	0.00	0.00	0.00
C22:6n3	22.14	22.00	22.07	0.10
C24:1n9	0.25	0.25	0.25	0.00
Sum	100.00	100.00		

Saturated	9.09	9.10	9,09	0.00
Monosaturated	19.23	19.26	19,25	0.02
Polysaturated	71.68	71.64	71,66	0.03
Omega-3	67.22	67.17	67,19	0.03

Table C5: An overview of the results from determination of fatty acid composition in the TG90 fish oil by GC-FID analysis.

TG90 Fatty acid	I	II	MEAN	SD
C14:0	0.22	0.22	0.22	0.00
C14:1	0.01	0.02	0.01	0.00
C15:0	0.03	0.03	0.03	0.00
C16:0	3.27	3.27	3.27	0.00
C16:1	0.97	0.97	0.97	0.00
C17:0	0.14	0.14	0.14	0.00
C17:1	0.12	0.14	0.13	0.01
C18:0	5.93	5.91	5.92	0.01
C18:1n11+n9	11.30	11.29	11.29	0.00
C18:1n7	3.55	3.51	3.53	0.03
C18:2n6	1.21	1.20	1.21	0.00
C18:3n6	0.24	0.24	0.24	0.00
C18:3n3	0.91	0.92	0.92	0.01
c18:4n3	3.12	3.13	3.12	0.00
C20:0	0.58	0.57	0.57	0.01
C20:1	2.81	2.79	2.80	0.01
C20:2n6	0.39	0.39	0.39	0.00
c20:3n6	0.37	0.37	0.37	0.00
C20:4n6	0.08	0.09	0.08	0.00
C20:3n3	0.29	0.29	0.29	0.00
c20:4n3	1.64	1.64	1.64	0.00
C20:5n3	35.01	35.04	35.03	0.02
C22:0	0.25	0.24	0.24	0.00
c22:1n11	0.51	0.50	0.51	0.01
C22:1n9	0.23	0.23	0.23	0.00
C22:2	1.43	1.44	1.43	0.01
C22:3	0.13	0.14	0.13	0.01
C22:4	0.51	0.55	0.53	0.03
c22:5n3	2.99	2.98	2.99	0.01
C24:0	0.00	0.00	0.00	0.00
C22:6n3	21.51	21.52	21.51	0.01
C24:1n9	0.26	0.25	0.26	0.01
Sum	100.00	100.00		

Saturated	10.41	10.37	10.39	0.03
Monosaturated	19.76	19.70	19.73	0.04
Polysaturated	69.83	69.93	69.88	0.07
Omega-3	65.47	65.51	65.49	0.03

Appendix D: Experimental data from the Schaal oven test of the TRAN oil experiment

TABLE D1: WEIGHT INCREASE DATA FOR THE COD LIVER OIL (TRAN) STORED AT 10 DEGREES, GIVEN IN G/100G.

STORAGE TIME (HOURS):	2	4	6	15.5	22.5	39.5	46.5	111.5	118.5	135.5	142.5	159.5	166.5	183.5	190.5	209
Parallel																
1	0,001															
2	0,004	0,004	-0,001													
3	0,040	0,006	0,001	0,009	0,003											
4	0,024	0,010	0,001	0,009	0,006	0,009	0,023									
5	0,004	0,004	0,004	0,004	0,001	0,006	0,017	0,036	0,033							
6	0,004	0,006	0,001	0,003	0,000	0,003	0,019	0,030	0,031	0,041	0,053					
7	0,001	0,003	-0,001	0,000	0,000	0,000	0,016	0,026	0,029	0,037	0,046	0,057	0,051	0,071	0,071	0,071
8	0,003	0,004	0,001	0,003	0,001	0,001	0,019	0,029	0,030	0,040	0,046	0,057	0,050	0,067	0,067	0,067
MEAN	0,010	0,005	0,001	0,005	0,002	0,004	0,019	0,030	0,031	0,039	0,048	0,057	0,051	0,069	0,069	0,069
SD	0,013	0,002	0,002	0,003	0,002	0,003	0,002	0,004	0,002	0,002	0,003	0,000	0,001	0,002	0,002	0,002

TABLE D2: WEIGHT INCREASE DATA FOR THE COD LIVER OIL (TRAN) STORED AT 22.5 DEGREES, GIVEN IN G/100G.

STORAGE TIME:	**4**	**6**	**23,5**	**30,5**	**96**	**105,5**	**120**	**129,5**	**143,5**	**153**	**167**	**174**	**192,5**	**199,5**	**288,5**	**294,5**	**336,5**	**361,5**	**368,5**
PARALLEL																			
1	0,000	-0,009																	
2	0,001	0,003	0,003	0,009															
3	0,000	0,001	0,000	0,009	0,020														
4	0,000	-0,001	-0,001	0,004	0,014	0,020	0,030												
5	0,000	0,001	0,003	0,010	0,021	0,027	0,037	0,039	0,050										
6	0,003	0,001	0,001	0,009	0,019	0,024	0,034	0,037	0,049	0,056	0,066								
7	0,000	0,004	0,004	0,009	0,019	0,027	0,036	0,037	0,046	0,056	0,066	0,070	0,086						
8	0,001	0,001	0,003	0,010	0,020	0,023	0,034	0,037	0,044	0,054	0,063	0,066	0,083	0,084	0,129				
9	0,000	0,000	0,003	0,010	0,026	0,024	0,041	0,041	0,049	0,059	0,071	0,071	0,089	0,089	0,133	0,140	0,170	0,197	
10	0,004	0,004	0,006	0,016	0,027	0,029	0,041	0,043	0,053	0,063	0,073	0,073	0,091	0,091	0,137	0,143	0,176	0,200	0,200
MEAN	0,001	0,001	0,002	0,009	0,021	0,025	0,036	0,039	0,048	0,057	0,068	0,070	0,087	0,088	0,133	0,141	0,173	0,198	0,200
SD	0,002	0,004	0,002	0,003	0,004	0,003	0,004	0,002	0,003	0,003	0,004	0,003	0,004	0,004	0,004	0,002	0,004	0,002	0,000

TABLE: D3: WEIGHT INCREASE DATA FOR THE COD LIVER OIL (TRAN) STORED AT 30 DEGREES, GIVEN IN G/100G.

Storage time (hours):	2.5	19	25	44	51	71.5	116	123	140	147	164	171	189
Parallels													
1	0,009996	0,022849	0,021421	0,038558	0,057122	0,094252	0,098536	0,115673	0,129954	0,14709	0,155659	0,17708	0,195644
2	-0,0057	0,009972	0,008548	0,022794									
3	0,008579	0,018588	0,025738	0,030027	0,047186	0,087223	0,095802	0,11296	0,12011	0,142988	0,148707	0,173015	0,195893
4	-0,00429	0,017149	0,01572	0,028581	0,044301	0,085744	0,092889	0,108609	0,120041	0,140048	0,145764	0,170058	0,187207
MEAN	0,002148	0,01714	0,017857	0,02999	0,049536	0,089073	0,095742	0,112414	0,123368	0,143375	0,150043	0,173384	0,192915
SD	0,007175	0,004639	0,006443	0,005639	0,005492	0,003712	0,002306	0,00291	0,004657	0,002888	0,004148	0,002878	0,004037

STORAGE TIME (HOURS):	**195**	**212**	**219**	**284**	**291**	**309**	**315**	**332**	**339**	**356**
Parallels										
1	0,195644	0,225634	0,238486	1,538022	1,746519	2,267762	2,449125	2,991789	3,203142	3,704391289
2										
3	0,195893	0,221631	0,2345	1,424159	1,635781	2,154827	2,334992	2,875486	3,094258	3,599004804
4	0,187207	0,217217	0,232937	1,409055	1,619127	2,13216	2,317938	2,903853	3,086773	3,584085972
MEAN	0,192915	0,221494	0,235308	1,457079	1,667142	2,184916	2,367352	2,923709	3,128057	3,629160689
SD	0,004037	0,003437	0,002336	0,057567	0,056538	0,059307	0,05824	0,049513	0,053181	0,053543598

Table D4: Weight increase data for the cod liver oil (TRAN) stored at 40 degrees, given in g/100g.

STORAGE TIME (HOURS):	**4**	**6**	**23.5**	**30.5**	**96**	**105.5**	**120**	**129.5**	**143.5**	**153**	**167**	**174**	**192.5**	**199.5**	**288.5**	**294.5**	**336.5**	**361.5**	**368.5**
Parallels																			
1	0,000	0,004																	
2	-0,004	-0,001	0,016	0,026															
3	0,007	0,003	0,024	0,034	0,179														
4	0,004	0,000	0,014	0,027	0,173	0,240	0,729												
5	0,001	0,001	0,021	0,031	0,176	0,221	0,669	1,030	1,662										
6	0,003	0,001	0,021	0,031	0,180	0,233	0,714	1,085	1,742	2,182	2,821								
7	0,000	0,006	0,024	0,030	0,180	0,243	0,736	1,102	1,745	2,188	2,826	3,139	3,914						
8	0,000	0,001	0,020	0,031	0,169	0,217	0,627	0,985	1,625	2,066	2,724	3,045	3,838	4,044	6,365				
9	0,001	0,001	0,023	0,036	0,176	0,223	0,637	1,000	1,641	2,090	2,744	3,051	3,857	4,062	6,330	6,415	6,884	7,095	
10	0,011	-0,003	0,019	0,029	0,183	0,235	0,705	1,069	1,727	2,168	2,803	3,110	3,894	4,107	6,369	6,456	6,921	7,114	7,148
MEAN	0,002	0,001	0,020	0,031	0,177	0,230	0,688	1,045	1,690	2,139	2,784	3,086	3,876	4,071	6,354	6,436	6,902	7,104	7,148
SD	0,004	0,003	0,004	0,003	0,004	0,010	0,044	0,048	0,054	0,056	0,047	0,046	0,035	0,032	0,021	0,029	0,026	0,013	0,000

Table D5: Weight increase data for the cod liver oil (TRAN) stored at 50 degrees, given in g/100g.

STORAGE TIME (HOURS):	**4**	**6**	**15,5**	**22,5**	**39,5**	**46,5**	**111,5**	**118,5**	**135,5**	**142,5**	**159,5**	**166,5**	**183,5**	**190,5**	**209**
PARALLELS															
1	-0,004														
2	-0,001	-0,006	0,010												
3	0,001	-0,001	-0,183	-0,163	-0,081										
4	-0,001	-0,004	0,021	0,049	0,130	0,197	3,846								
5	-0,004	-0,003	0,020	0,040	0,127	0,193	3,865	4,121	4,660						
6	0,000	0,001	0,037	0,061	0,163	0,378	4,176	4,400	4,892	5,054	5,385				
7	0,000	0,001	0,039	0,069	0,183	0,489	4,221	4,461	4,956	5,114	5,440	5,553	5,775	5,852	6,036
8	0,061	0,036	0,057	0,080	0,153	0,204	3,970	4,247	4,824	5,012	5,386	5,513	5,770	5,858	6,057
MEAN	0,006	0,003	0,000	0,023	0,112	0,292	4,015	4,307	4,833	5,060	5,404	5,533	5,772	5,855	6,047
SD	0,021	0,013	0,076	0,084	0,089	0,121	0,156	0,133	0,110	0,042	0,026	0,020	0,002	0,003	0,010

Appendix E: Experimental data from the peroxide value measurements

Table E1: PV results for TRAN fish oil samples stored

at 10 degrees.

SAMPLE	MEAN	SD	STORAGE TIME (HOURS)
10-1	2,0351	0,0801	4
10-2	3,2926	0,1617	15,5
10-3	7,7659	0,4219	39,5
10-4	22,6911	2,5573	111,5
10-5	33,5528	0,1355	135,5
10-6	27,1514	2,3055	159,5
10-7	36,3200	2,2715	209
10-8	52,6318	1,5282	279,5

Table E2: PV results for TRAN fish oil samples stored

at 22.5 degrees.

SAMPLE	MEAN	SD	STORAGE TIME (HOURS)
20-1	5,8496	0,9321	6
20-2	11,0819	2,4134	30,5
20-3	26,9920	0,8526	96
20-4	29,5697	1,4661	120
20-5	37,2084	0,9543	143,5
20-6	43,0739	5,1796	167
20-7	50,9953	0,8467	192,5
20-8	70,5371	2,7297	288,5
20-9	101,3974	2,5395	361,5
20-10	98,6502	2,4279	368,5

Table E3: PV results for TRAN fish oil samples stored

at 30 degree

SAMPLE	MEAN	SD	STORAGE TIME (HOURS)
30-1	2,0076	0,0239	4
30-2	4,5639	0,0000	15,5
30-3	13,6652	2,8308	39,5
30-4	43,2877	3,5530	111,5
30-5	47,2148	0,9543	135,5
30-6	56,7816	6,3196	159,5
30-7	83,5337	5,9684	209
30-8	x	x	279,5

Table E4: PV results for TRAN fish oil samples stored

at 40 degrees

SAMPLE	MEAN	SD	STORAGE TIME(HOURS)
40-1	15,1851	4,5717	6
40-2	29,5991	1,1877	30,5
40-3	164,7470	23,1293	96
40-4	448,9254	49,6429	120
40-5	695,2691	26,7176	143,5
40-6	1009,0896	96,0146	167
40-7	1587,1579	49,6429	192,5
40-8	1274,8579	347,2794	288,5
40-9	976,3198	145,3441	361,5
40-10	992,4881	152,1929	368,5

Table E5: PV results for TRAN fish oil samples stored

at 50 degrees.

SAMPLE	MEAN	SD	STORAGE TIME (HOURS)
50-1	3,0130	0,4946	4
50-2	33,3003	6,2608	15,5
50-3	82,0673	7,4788	39,5
50-4	865,7670	89,7376	111,5
50-5	953,4980	63,8584	135,5
50-6	1144,5380	198,4035	159,5
50-7	664,8261	12,2579	209
50-8	916,5732	113,4920	279,5

Appendix F: Experimental data from the TBARS assay

Table F1: TBARS results for TRAN fish oil samples stored at 10 degrees.

SAMPLE	TBARS	SD	STORAGE TIME (HOURS)
10-1	1,177	0,1355	4
10-2	1,536	0,0633	15,5
10-3	3,029	0,0140	39,5
10-4	7,140	0,2506	111,5
10-5	8,648	0,2058	135,5
10-6	9,399	0,6213	159,5
10-7	14,363	0,5341	209
10-8	21,334	0,8593	279,5

Table F2: TBARS results for TRAN fish oil samples stored at 22.5 degrees.

SAMPLE	TBARS	SD	STORAGE TIME (HOURS)
20-1	5,9357	0,1291	6
20-2	8,4871	0,3735	30,5
20-3	21,1842	1,124	96
20-4	26,7306	1,0228	120
20-5	34,917	1,5854	143,5
20-6	42,4602	0,7415	167
20-7	53,0447	0,7106	192,5
20-8	105,38	3,6791	288,5
20-9	107,96	4,922	361,5
20-10	104,84	5,3955	368,5

Table F3: TBARS results for TRAN fish oil samples stored at 30 degrees.

SAMPLE	TBARS	SD	STORAGE TIME (HOURS)
30-1	1,0536	0,1353	4
30-2	1,9076	0,0285	15,5
30-3	5,2486	0,0613	39,5
30-4	17,9056	1,0888	111,5
30-5	24,14	4,8937	135,5
30-6	27,1447	2,3265	159,5
30-7	40,096	4,4842	209
30-8	163,7858	14,4487	279,5

Table F4: TBARS results for TRAN fish oil samples stored at 40 degrees.

SAMPLE	TBARS	SD	STORAGE TIME (HOURS)
40-1	7,5846	0,3413	6
40-2	24,2722	0,9598	30,5
40-3	96,8686	0,7032	96
40-4	352,3913	7,0854	120
40-5	608,34	6,6671	143,5
40-6	971,4	32,9906	167
40-7	1246,15	31,3479	192,5
40-8	1230,18	68,8784	288,5
40-9	1017,8	27,6964	361,5
40-10	1052,15	73,2776	368,5

Table F5: TBARS results for TRAN fish oil samples stored at 50 degrees.

SAMPLE	TBARS	SD	STORAGE TIME (HOURS)
50-1	1,3674	0,0636	4
50-2	8,8579	0,9814	15,5
50-3	30,4774	0,97	39,5
50-4	338,8188	4,0762	111,5
50-5	351,7764	27,6841	135,5
50-6	294,6768	20,9697	159,5
50-7	235,3338	12,542	209
50-8	160,9787	9,0326	279,5

Appendix G: Experimental data from the Schaal oven tests performed on the TRAN fish samples added with different antioxidant additives

Table G1: Weight increase data given in g/100g for TRAN fish oil samples added with a mixed tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**2**	**19,5**	**23,5**	**26**	**43**	**50**	**67**	**73**	**139**	**146**	**163**	**170**	**187**	**194**	**211**	**218**	**235**	**242**
PARALLELS																		
1	-0,0157	0,0271	0,0541	0,0541	0,1895	X												
2	0,0114	0,0686	0,0886	0,0943	0,2444	0,3044	0,4702	0,5317	2,4469	2,7342	3,5375	3,7790	4,4136	4,6009	5,0997	5,2183	5,5671	5,6328
3	0,0014	0,0500	0,0801	0,1001	0,2316	0,2959	0,4475	0,5046	2,0815	2,3445	3,1536	3,3981	X					
MEAN	-0,0009	0,0486	0,0743	0,0828	0,2218	0,3002	0,4588	0,5182	2,2642	2,5394	3,3456	3,5886	4,4136	4,6009	5,0997	5,2183	5,5671	5,6328
SD	0,0112	0,0170	0,0147	0,0204	0,0235	0,0043	0,0114	0,0135	0,1827	0,1949	0,1919	0,1905	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000

Table G2: Weight increase data given in g/100g for TRAN fish oil samples added with a α-tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**2**	**19,5**	**23,5**	**26**	**43**	**50**	**67**	**73**	**139**	**146**	**163**	**170**	**187**	**194**	**211**	**218**	**235**	**242**
PARALLELS																		
1	0,0028	0,0712	0,1069	0,1269	0,2980	0,3636	0,6487	1,0009	4,6969	4,8994	5,3614	5,5040	5,7621	5,8790	6,1471	6,2070	6,3553	6,3895
2	0,0042	0,1068	0,1367	0,1610	0,3505	0,4260	1,1284	1,5274	4,8729	5,0438	5,4129	5,5397	5,7819	5,8460	6,0697	6,1267	6,2606	6,2834
3	0,0028	0,0872	0,1087	0,1387	0,3089	0,3761	0,7037	1,0469	4,6226	4,8186	5,2677	5,4064	5,6653	5,7769	6,0472	6,1116	6,2675	6,2932
MEAN	0,0014	0,0884	0,1174	0,1422	0,3191	0,3885	0,8269	1,1917	4,7308	4,9206	5,3473	5,4834	5,7364	5,8340	6,0886	6,1484	6,2945	6,3221
SD	0,0030	0,0145	0,0136	0,0141	0,0226	0,0269	0,2143	0,2380	0,1049	0,0931	0,0600	0,0563	0,0509	0,0425	0,0427	0,0418	0,0430	0,0478

Table G3: Weight increase data given in g/100g for TRAN fish oil samples added with a AP antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**2**	**19,5**	**23,5**	**26**	**43**	**50**	**67**	**73**	**139**	**146**	**163**	**170**	**187**	**194**	**211**	**218**	**235**	**242**
PARALLELS																		
1	-0,0300	-0,0172	-0,0043	-0,0029	0,0900	0,1429	1,0605	1,4736	4,9896	5,1683	5,6014	5,7114	5,9473	6,0344	6,2517	6,2974	6,4461	6,4718
2	-0,0257	-0,0043	0,0114	0,0228	0,1482	0,4889	1,6561	2,0965	5,1423	5,2677	5,6226	5,7138	5,9205	5,9576	6,1671	6,2155	6,3438	6,3595
3	-0,0300	-0,0143	0,0043	0,0143	0,1070	0,1769	1,1840	1,5905	4,9041	5,0596	5,4533	5,5603	5,7657	5,8912	6,1038	6,1523	6,2964	6,3178
MEAN	-0,0285	-0,0119	0,0038	0,0114	0,1151	0,2696	1,3002	1,7202	5,0120	5,1652	5,5591	5,6619	5,8778	5,9611	6,1742	6,2217	6,3621	6,3830
SD	0,0020	0,0055	0,0064	0,0107	0,0244	0,1557	0,2567	0,2704	0,0985	0,0850	0,0753	0,0718	0,0800	0,0585	0,0606	0,0594	0,0625	0,0651

Table G4: Weight increase data given in g/100g for TRAN fish oil samples added with a A/L/T antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**2**	**19,5**	**23,5**	**26**	**43**	**50**	**67**	**73**	**139**	**146**	**163**	**170**	**187**	**194**	**211**	**218**	**235**	**242**
PARALLELS																		
1	-0,0029	0,0071	0,0057	0,0128	0,0527	0,0713	0,1668	0,2067	3,7109	3,9133	4,4080	4,5705	4,9041	5,0039	5,3318	5,4131	5,6198	5,6597
2	0,0043	0,0100	0,0100	0,0071	0,0656	0,1013	0,2155	0,4452	3,9829	4,1712	4,6379	4,7777	5,0574	5,1687	5,4713	5,5469	5,7353	5,7767
3	-0,0014	0,0000	0,0000	0,0029	0,0543	0,0757	0,1714	0,2443	3,8102	4,0174	4,5074	4,6603	4,9417	5,1074	5,4103	5,4860	5,6946	5,7446
MEAN	0,0000	0,0057	0,0052	0,0076	0,0576	0,0828	0,1846	0,2988	3,8347	4,0340	4,5178	4,6695	4,9678	5,0934	5,4045	5,4820	5,6832	5,7270
SD	0,0031	0,0042	0,0041	0,0041	0,0058	0,0132	0,0219	0,1047	0,1124	0,1059	0,0941	0,0848	0,0652	0,0680	0,0571	0,0547	0,0478	0,0493

Table G5: Weight increase data given in g/100g for TRAN fish oil samples added with a rosemary extract antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**19,5**	**23,5**	**26**	**43**	**50**	**67**	**73**	**139**	**146**	**163**	**170**	**187**	**194**	**211**	**218**	**235**	**242**
PARALLELS																	
1	-0,0357	-0,0328	-0,0342	-0,0243	-0,0185	0,0057	0,0271	2,6695	2,9977	3,7852	4,0121	4,5143	4,6784	5,1350	5,2363	5,5231	5,5887
2	-0,0300	-0,0343	-0,0271	-0,0228	-0,0071	0,0357	0,0628	3,2982	3,5894	4,3047	4,5032	4,9758	5,1129	5,5198	5,6069	5,8667	5,9138
3	-0,0357	-0,0314	-0,0271	-0,0214	-0,0228	-0,0057	0,0185	2,5335	2,8915	3,7845	4,0299	4,5848	4,7617	5,2781	5,3822	5,6975	5,7574
MEAN	-0,0338	-0,0328	-0,0295	-0,0228	-0,0162	0,0119	0,0362	2,8337	3,1595	3,9582	4,1817	4,6916	4,8510	5,3110	5,4085	5,6958	5,7533
SD	0,0027	0,0012	0,0034	0,0012	0,0066	0,0175	0,0192	0,3331	0,3070	0,2451	0,2274	0,2030	0,1883	0,1588	0,1524	0,1403	0,1328

Table G6: Weight increase data given in g/100g for TRAN fish oil samples added with a BHA antioxidant additive (conc. = 150 ppm) and stored at 50 degrees.

STORAGE TIME	**2**	**19,5**	**23,5**	**26**	**43**	**50**	**67**	**73**	**139**	**146**	**163**	**170**	**187**	**194**	**211**	**218**	**235**	**242**
PARALLELS																		
1	0,0100	0,0385	0,0528	0,0599	0,1341	0,1712	0,3095	0,3737	2,7999	3,1151	3,9552	4,1906	4,7226	4,9009	5,3701	5,4628	5,7695	5,8280
2	0,0043	0,0271	0,0443	0,0486	0,1357	0,1814	0,3314	0,3928	3,0822	3,3821	4,1690	4,3833	4,8646	5,0289	5,4873	5,5830	5,8744	5,9186
3	0,0114	0,0342	0,0456	0,0570	0,1354	0,1853	0,3093	0,3749	2,6842	2,9907	3,7947	4,0114	4,5061	4,7128	5,1504	5,2602	5,5638	5,6208
MEAN	0,0086	0,0333	0,0476	0,0552	0,1351	0,1793	0,3167	0,3805	2,8554	3,1626	3,9730	4,1951	4,6977	4,8808	5,3359	5,4353	5,7359	5,7891
SD	0,0031	0,0047	0,0037	0,0048	0,0007	0,0060	0,0103	0,0087	0,1671	0,1633	0,1533	0,1519	0,1474	0,1298	0,1397	0,1332	0,1290	0,1247

Appendix H: Experimental data from the Schaal oven tests performed on the 18/12-S fish samples added with different antioxidant additives

Table H1: Weight increase data given in g/100g for 18/12-S fish oil samples without addition of antioxidant additive stored at 50 degrees

STORAGE TIME	**16,5**	**22,5**	**41,5**	**43,5**	**46,5**	**48,5**	**69**	**113**	**120**	**137**	**144**	**161**	**168**	**186**	**192**	**209**	**216**	**281**
PARALLELS																		
1	0,0443	0,0700	0,7545	0,8145	1,0102	1,1245	2,7906	5,6299	5,8942	6,4486	6,5886	6,7816	6,8258	6,8916	6,9001	6,9130	6,9330	6,9316
2	0,0371	0,0855	1,0663	1,1291	1,3543	1,5011	3,2460	5,9361	6,1599	6,5762	6,6817	6,8257	6,8570	6,9041	6,9169	6,9269	6,9426	6,9269
3	0,0557	0,0928	1,0090	1,0675	1,2873	1,4087	3,1213	5,8187	6,0442	6,5023	6,6237	6,7678	6,8106	6,8663	6,8905	6,8991	6,9162	6,9120
MEAN	0,0457	0,0828	0,9433	1,0037	1,2173	1,3448	3,0527	5,7949	6,0328	6,5091	6,6313	6,7917	6,8312	6,8873	6,9025	6,9130	6,9306	6,9235
SD	0,0077	0,0095	0,1355	0,1361	0,1489	0,1602	0,1921	0,1262	0,1088	0,0523	0,0384	0,0247	0,0193	0,0157	0,0109	0,0113	0,0109	0,0084

Table H2: Weight increase data given in g/100g for 18/12-S fish oil samples added with a mixed tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees

STORAGE TIME	**4**	**21**	**28**	**45**	**51,5**	**71,5**	**76**	**142**	**147**
PARALLELS									
1	-0,0056	0,0253	0,0857	0,2064	0,2739	0,4958	0,5688	3,6669	3,9380
2	0,0070	0,0479	0,0958	0,2452	0,3171	0,5469	0,6328	3,9366	4,2044
3	-0,0028	0,0227	0,0710	0,1945	0,2654	0,4741	0,5436	3,5997	3,8779
MEAN	-0,0005	0,0320	0,0842	0,2154	0,2855	0,5056	0,5818	3,7344	4,0068
SD	0,0054	0,0113	0,0102	0,0217	0,0226	0,0305	0,0375	0,1456	0,1419

Table H3: Weight increase data given in g/100g for 18/12-S fish oil samples added with a α-tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**4**	**21**	**28**	**45**	**51,5**	**71,5**	**76**	**142**	**147**
PARALLELS									
1	0,0057	0,0510	0,0950	0,2864	0,3573	0,7854	1,0604	5,5573	5,7373
2	0,0086	0,0527	0,0969	0,2908	0,3607	0,7784	1,0407	5,5430	5,7340
3	0,0130	0,0493	0,1029	0,2797	0,3536	0,7419	1,0013	5,6194	5,8063
MEAN	0,0091	0,0510	0,0983	0,2856	0,3572	0,7686	1,0341	5,5732	5,7592
SD	0,0030	0,0014	0,0034	0,0046	0,0029	0,0191	0,0246	0,0332	0,0333

Table H4: Weight increase data given in g/100g for 18/12-S fish oil samples added with a A/L/T antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**4**	**21**	**28**	**45**	**51,5**	**71,5**	**76**	**142**	**147**
PARALLELS									
1	-0,0014	0,0415	0,0595	0,3014	0,6028	1,9301	2,2508	5,7819	5,9133
2	0,0028	0,0384	0,0641	0,2904	0,5894	1,9690	2,2879	5,8912	6,0265
3	0,0028	0,0340	0,0610	0,2398	0,5036	1,8343	2,1436	5,8448	5,9725
MEAN	0,0014	0,0380	0,0615	0,2772	0,5653	1,9111	2,2274	5,8393	5,9707
SD	0,0020	0,0030	0,0019	0,0269	0,0439	0,0566	0,0612	0,0448	0,0462

Table H5: Weight increase data given in g/100g for 18/12-S fish oil samples added with a rosemary extract antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**21**	**28**	**45**	**51,5**	**71,5**	**76**	**142**	**147**
PARALLELS								
1	-0,0155	-0,0169	0,0042	0,0155	0,0917	0,1340	4,4196	4,6509
2	0,0000	-0,0071	0,0257	0,0328	0,1185	0,1742	4,6768	4,9096
3	-0,0014	-0,0086	0,0200	0,0300	0,1172	0,1729	4,6330	4,8574
MEAN	-0,0056	-0,0109	0,0166	0,0261	0,1091	0,1604	4,5765	4,8060
SD	0,0070	0,0043	0,0091	0,0076	0,0124	0,0187	0,1124	0,1117

Table H6: Weight increase data given in g/100g for 18/12-S fish oil samples added with a BHA antioxidant additive (conc. = 150 ppm) and stored at 50 degrees.

STORAGE TIME	**4**	**21**	**28**	**45**	**51,5**	**71,5**	**76**	**142**	**147**
PARALLELS									
1	0,0030	0,0546	0,0865	0,2702	0,3628	0,7347	0,8683	5,5526	5,7575
2	-0,0057	0,0525	0,0823	0,2513	0,3279	0,6459	0,7495	4,8803	5,0989
3	0,0015	0,0552	0,0798	0,1408	0,3411	0,6720	0,7750	5,1378	5,3526
MEAN	-0,0004	0,0541	0,0829	0,2207	0,3439	0,6842	0,7976	5,1902	5,4030
SD	0,0038	0,0011	0,0028	0,0571	0,0144	0,0373	0,0510	0,2770	0,2712

Appendix I: Experimental data from the Schaal oven tests performed on the 18/12-B fish samples added with different antioxidant additives

Table I1: Weight increase data given in g/100g for 18/12-B fish oil samples without addition of antioxidant additive stored at 50 degrees

STORAGE TIME	**16,5**	**22,5**	**41,5**	**43,5**	**46,5**	**48,5**	**69**	**113**	**120**	**137**	**144**	**161**	**168**	**186**	**192**	**209**	**216**	**281**	**288**
PARALLELS																			
1	0,0656	0,1468	1,2288	1,2815	1,5067	1,6692	3,4767	6,2664	6,4930	6,8751	6,9378	7,0233	7,0404	7,0732	7,0917	7,1088	7,1274	7,1388	7,1502
2	-0,2051	-0,0883	1,2760	1,3529	1,6035	1,7516	3,5958	6,2276	6,4227	6,6690	6,7118	6,7687	6,7815	6,8385	6,8243	6,8314	6,8556	6,8399	6,8527
3	-0,6180	-0,5117	0,7399	0,8079	1,0461	1,1807	2,9327	5,5833	5,7902	6,1049	6,1786	6,2438	6,2679	6,3019	6,3118	6,3104	6,3331	6,3359	6,3388
MEAN	-0,2525	-0,1511	1,0816	1,1474	1,3854	1,5339	3,3351	6,0257	6,2353	6,5497	6,6094	6,6786	6,6966	6,7379	6,7426	6,7502	6,7720	6,7715	6,7806
SD	0,2811	0,2725	0,2424	0,2418	0,2432	0,2520	0,2887	0,3133	0,3160	0,3256	0,3183	0,3246	0,3211	0,3228	0,3236	0,3310	0,3296	0,3313	0,3352

Table I2: Weight increase data given in g/100g for 18/12-B fish oil samples added with a mixed tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50degrees.

STORAGE TIME	**5**	**23**	**29**	**46**	**53**	**73,5**	**77**	**142**	**149**	**166**	**173**	**190**	**197**	**214**	**221**	**238**	**245**	**310**
PARALLELS																		
1	-0,0071	-0,0043	0,0171	0,1323	0,2049	0,4609	0,4424	3,0970	3,5408	4,7472	5,1000	6,0133	6,3078	6,8370	6,9366	7,0817	7,1044	7,2296
2	-0,0186	0,0614	0,1028	0,2756	0,3756	0,6997	0,6811	4,3881	4,7922	5,8218	6,1074	6,7671	6,9156	7,1184	7,1584	7,2055	7,2141	7,2626
3	-0,0057	0,0000	0,0256	0,1382	0,2237	0,4773		3,1718	3,6263	4,8403	5,1951	6,0742	6,3449	6,8379	6,9306	7,0574	7,0730	7,1614
MEAN	-0,0105	0,0190	0,0485	0,1820	0,2680	0,5460	0,5618	3,5523	3,9865	5,1364	5,4675	6,2849	6,5228	6,9311	7,0085	7,1148	7,1305	7,2179
SD	0,0058	0,0300	0,0386	0,0662	0,0764	0,1089	0,1194	0,5918	0,5708	0,4861	0,4541	0,3419	0,2782	0,1324	0,1060	0,0649	0,0604	0,0422

Table I3: Weight increase data given in g/100g for 18/12-B fish oil samples added with a α-tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**5**	**23**	**29**	**46**	**53**	**73,5**	**77**	**142**	**149**	**166**	**173**	**190**	**197**	**214**	**221**	**238**	**245**	**310**
PARALLELS																		
1	-0,0071	-0,0043	0,0100	0,1683	0,2495	0,5290	5,0748	5,4427	6,3068	6,5477	6,9370	7,0283	7,1538	7,1709	7,2008	7,2037	7,2193	-0,0071
2	-0,0128	-0,0100	-0,0014	0,1641	0,2555	0,5281	5,1857	5,5640	6,4304	6,6502	6,9956	7,0613	7,1598	7,1912	7,2112	7,2155	7,2397	-0,0128
3	-0,0100	-0,0014	0,0214	0,1952	0,2893	0,5814	5,3341	5,6833	6,4756	6,6879	6,9929	7,0556	7,1411	7,1639	7,1768	7,1796	7,1867	-0,0100
MEAN	-0,0100	-0,0052	0,0100	0,1759	0,2648	0,5462	5,1982	5,5633	6,4043	6,6286	6,9752	7,0484	7,1516	7,1753	7,1962	7,1996	7,2153	-0,0100
SD	0,0023	0,0036	0,0093	0,0138	0,0175	0,0249	0,1062	0,0982	0,0714	0,0592	0,0270	0,0144	0,0078	0,0116	0,0144	0,0149	0,0218	0,0023

Table I4: Weight increase data given in g/100g for 18/12-B fish oil samples added with a A/L/T antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	5	23	29	46	53	73,5	77	142	149	166	173	190	197	214	221	238	245	310
PARALLELS																		
1	-0,0071	-0,0085	0,0114	0,1025	0,2207	1,5309	1,6562	5,9471	6,1678	6,6207	6,7147	6,8471	6,8970	6,9568	6,9767	6,9953	6,9967	7,0337
2	-0,0014	0,0000	0,0157	0,1171	0,2356	1,5437	1,6708	5,9662	6,2047	6,6588	6,7530	6,8844	6,9387	7,0115	7,0300	7,0486	7,0515	7,0929
3	-0,0014	-0,0171	0,0157	0,1256	0,2512	1,5142	1,6327	5,8957	6,1369	6,6250	6,7235	6,8876	6,9318	7,0118	7,0289	7,0560	7,0531	7,1188
MEAN	-0,0033	-0,0086	0,0143	0,1151	0,2358	1,5296	1,6532	5,9363	6,1698	6,6348	6,7304	6,8730	6,9225	6,9933	7,0119	7,0333	7,0338	7,0818
SD	0,0027	0,0070	0,0020	0,0095	0,0124	0,0120	0,0157	0,0298	0,0277	0,0170	0,0164	0,0184	0,0182	0,0258	0,0249	0,0271	0,0262	0,0356

Table I5: Weight increase data given in g/100g for 18/12-B fish oil samples added with a rosemary extract antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	23	29	46	53	73,5	77	142	149	166	173	190	197	214	221	238	245	310
PARALLELS																	
1	0,0014	0,0214	0,2086	0,4372	2,0017	2,1260	6,4552	6,6510	6,9324	6,9753	7,0810	7,0982	7,1382	7,1525	7,1424	7,1410	7,1696
2	0,0014	0,0214	0,2126	0,4509	2,0560	2,1958	6,4677	6,6446	6,9057	6,9457	7,0384	7,0627	7,0940	7,1097	7,1040	7,1055	7,1340
3	0,0057	0,0185	0,2009	0,4076	1,9924	2,1235	6,5129	6,6996	6,9733	7,0132	7,1144	7,1272	7,1571	7,1699	7,1600	7,1585	7,1685
MEAN	0,0029	0,0205	0,2074	0,4319	2,0167	2,1484	6,4786	6,6651	6,9371	6,9780	7,0779	7,0960	7,1298	7,1440	7,1355	7,1350	7,1574
SD	0,0020	0,0014	0,0048	0,0181	0,0281	0,0335	0,0248	0,0246	0,0278	0,0276	0,0311	0,0264	0,0264	0,0253	0,0234	0,0221	0,0165

Table I6: Weight increase data given in g/100g for 18/12-B fish oil samples added with a BHA antioxidant additive (conc. = 150 ppm) and stored at 50 degrees.

STORAGE TIME	5	23	29	46	53	73,5	77	142	149	166	173	190	197	214	221	238	245	310
PARALLELS																		
1	-0,006	0,018	0,060	0,250	0,341	0,762	5,491	5,827	6,600	6,755	7,023	7,089	7,173	7,186	7,197	7,195	7,206	-0,006
2	-0,001	0,027	0,061	0,244	0,331	0,759	5,498	5,839	6,612	6,787	7,043	7,100	7,183	7,200	7,209	7,216	7,222	-0,001
3	-0,003	0,027	0,057	0,245	0,332	0,759	5,526	5,869	6,644	6,814	7,086	7,148	7,236	7,253	7,263	7,272	7,278	-0,003
MEAN	-0,0033	0,0242	0,0594	0,2465	0,3349	0,7601	5,5050	5,8451	6,6190	6,7852	7,0508	7,1125	7,1976	7,2133	7,2232	7,2275	7,2351	-0,0033
SD	0,0018	0,0041	0,0018	0,0027	0,0045	0,0014	0,0149	0,0179	0,0185	0,0239	0,0266	0,0253	0,0276	0,0289	0,0287	0,0326	0,0307	0,0018

Appendix J: Experimental data from the Schaal oven tests performed on the TG60 fish samples added with different antioxidant additives

Table J1: Weight increase data given in g/100g for TG60 fish oil samples without addition of antioxidant additive stored at 50 degrees

STORAGE TIME	**16,5**	**22,5**	**41,5**	**43,5**	**46,5**	**48,5**	**69**	**113**	**120**	**137**
PARALLELS										
1	1,4564	2,2517	4,8333	4,9689	5,3016	5,4744	7,0008	6,8209	6,8109	6,7595
2	1,5999	2,4291	5,1079	5,2449	5,5860	5,7715	7,0646	6,9147	6,9104	6,8705
3	1,4630	2,2901	5,0421	5,1690	5,5312	5,7066	7,2039	7,0556	7,0399	7,0014
MEAN	1,5064	2,3236	4,9944	5,1276	5,4730	5,6509	7,0897	6,9304	6,9204	6,8771
SD	0,0661	0,0762	0,1171	0,1164	0,1232	0,1276	0,0848	0,0965	0,0938	0,0989

Table J2: Weight increase data given in g/100g for TG60 fish oil samples added with a mixed tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,0972	0,1473	0,4861	0,7506	1,4225	2,5233	4,6750	6,8924	6,8580
2	0,1300	0,1957	0,6185	1,0156	2,2697	3,6038	5,6578	6,9304	6,8019
3	0,1372	0,1900	0,5915	0,9444	1,9374	3,2191	5,2265	6,9511	6,8596
MEAN	0,1215	0,1777	0,5654	0,9035	1,8765	3,1154	5,1864	6,9246	6,8399
SD	0,0174	0,0216	0,0571	0,1120	0,3485	0,4471	0,4022	0,0243	0,0269

Table J3: Weight increase data given in g/100g for TG60 fish oil samples added with a α-tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,1471	0,2071	0,7326	2,0479	3,8902	5,1312	6,8506	6,9549	6,8735
2	0,1730	0,2417	1,1483	2,6441	4,4646	5,6358	6,9471	6,8513	6,7441
3	0,1755	0,2383	1,2200	2,7096	4,5375	5,7460	7,0474	6,9603	6,8619
MEAN	0,1652	0,2290	1,0336	2,4672	4,2974	5,5043	6,9484	6,9222	6,8265
SD	0,0129	0,0156	0,2149	0,2977	0,2895	0,2677	0,0803	0,0501	0,0584

Table J4: Weight increase data given in g/100g for TG60 fish oil samples added with a rosemary extract antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,0758	0,3804	3,0307	4,3851	5,8483	6,7379	6,8523	6,7751	6,6893
2	0,0271	0,2993	3,0981	4,4861	5,9624	6,8075	6,8160	6,7376	6,6464
3	0,0029	0,2202	2,9911	4,3952	5,9165	6,8259	6,9317	6,8544	6,7729
MEAN	0,0352	0,3000	3,0400	4,4221	5,9091	6,7904	6,8667	6,7891	6,7029
SD	0,0303	0,0654	0,0441	0,0454	0,0469	0,0379	0,0483	0,0487	0,0525

Table J5: Weight increase data given in g/100g for TG60 fish oil samples added with a BHA antioxidant additive (conc. = 150 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,2227	0,3169	1,4333	2,8038	4,5598	5,7233	7,0653	7,0110	6,9268
2	0,2475	0,3633	1,8438	3,2985	4,9964	6,0534	6,4954	6,4124	6,3366
3	0,2595	0,3636	1,8664	3,2908	4,9761	6,0498	6,5174	6,4290	6,3520
MEAN	0,2432	0,3479	1,7145	3,1310	4,8441	5,9422	6,6927	6,6175	6,5385
SD	0,0153	0,0219	0,1990	0,2314	0,2012	0,1548	0,2636	0,2783	0,2746

Appendix K: Experimental data from the Schaal oven tests performed on the TG90 fish samples added with different antioxidant additives

Table K1: Weight increase data given in g/100g for TG90 fish oil samples without addition of antioxidant additive stored at 50 degrees

STORAGE TIME	**16,5**	**22,5**	**41,5**	**43,5**	**46,5**	**48,5**	**69**	**113**	**120**	**137**
PARALLELS										
1	0,3452	0,6305	3,1084	3,2553	3,6447	3,8701	6,3351	6,5320	6,5306	6,4821
2	0,3677	0,7196	3,4927	3,6253	4,0257	4,2523	6,5950	6,4953	6,4924	6,4483
3	0,3473	0,6674	3,2656	3,4000	3,7916	4,0260	6,4155	6,4455	6,4384	6,3898
MEAN	0,3534	0,6725	3,2889	3,4269	3,8207	4,0495	6,4486	6,4909	6,4871	6,4400
SD	0,0101	0,0366	0,1578	0,1522	0,1569	0,1569	0,1086	0,0354	0,0378	0,0381

Table K2: Weight increase data given in g/100g for TG90 fish oil samples added with a mixed tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,1001	0,1529	0,4760	0,7176	1,1535	1,7467	3,5864	6,2365	6,3694
2	0,1269	0,1740	0,5135	0,7702	1,2552	1,9584	3,9267	6,4471	6,3958
3	0,1100	0,1629	0,5087	0,7601	1,2473	1,9174	3,8520	6,3310	6,2824
MEAN	0,1123	0,1633	0,4994	0,7493	1,2187	1,8742	3,7884	6,3382	6,3492
SD	0,0111	0,0086	0,0167	0,0228	0,0462	0,0917	0,1461	0,0861	0,0484

Table K3: Weight increase data given in g/100g for TG90 fish oil samples added with a α-tocopherol antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,1556	0,2198	0,5452	0,9462	2,4104	3,7677	5,8299	6,4022	6,3365
2	0,1856	0,2370	0,6125	1,2906	3,0666	4,4458	6,4045	6,4816	6,4060
3	0,1671	0,2328	0,5970	1,1411	2,7664	4,1546	6,0912	6,2583	6,1883
MEAN	0,1694	0,2299	0,5849	1,1260	2,7478	4,1227	6,1085	6,3807	6,3103
SD	0,0124	0,0073	0,0288	0,1410	0,2682	0,2778	0,2349	0,0924	0,0908

Table K4: Weight increase data given in g/100g for TG90 fish oil samples added with a rosemary extract antioxidant additive (conc. = 1000 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	-0,1071	-0,1185	0,0571	0,3941	1,7362	3,1068	5,2427	6,1565	6,0780
2	-0,1085	-0,0985	0,1984	0,8978	2,7462	4,1464	6,0362	6,0790	5,9905
3	-0,1156	-0,1070	0,1499	0,7365	2,4763	3,9221	5,9488	6,1885	6,1058
MEAN	-0,1104	-0,1080	0,1351	0,6761	2,3195	3,7251	5,7425	6,1413	6,0581
SD	0,0037	0,0082	0,0586	0,2100	0,4270	0,4467	0,3552	0,0460	0,0491

Table K5: Weight increase data given in g/100g for TG90 fish oil samples added with a BHA antioxidant additive (conc. = 150 ppm) and stored at 50 degrees.

STORAGE TIME	**15,5**	**20,5**	**39,5**	**49,5**	**62,5**	**72,5**	**88,5**	**112,5**	**135,5**
PARALLELS									
1	0,1684	0,2326	0,7235	1,2386	2,7527	4,0755	6,0291	6,3673	6,2945
2	0,1882	0,2637	0,8282	1,5495	3,2387	4,5416	6,3050	6,3506	6,2679
3	0,1844	0,2573	0,8161	1,4865	3,0858	4,4051	6,2374	6,3703	6,2931
MEAN	0,1803	0,2512	0,7893	1,4249	3,0257	4,3407	6,1905	6,3627	6,2852
SD	0,0086	0,0134	0,0468	0,1342	0,2029	0,1957	0,1174	0,0087	0,0122

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The Stabilizing Effect of Antioxidants in Fish Oils

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