Master's thesis

NTNU
Norwegian University of Science and Technology
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Application of natural antioxidants and modified atmosphere packaging to prolong shelf life and enhance quality of fish products

Master's thesis in Biotechnology
Supervisor: Turid Rustad
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Preface

This study is a master thesis in the 5-year master program in Biotechnology, MBIOT5, at the Norwegian University of Life Science and Technology, NTNU. The research was performed at the Department of Biotechnology and Food Science and was carried out from fall 2020 to spring 2021.

I want to thank my supervisor Professor Turid Rustad for her knowledge, patience and giving me the opportunity to dwell deeper into a field of high interest to me. Working with her has been inspiring.

I would also like to thank Siri Stavrum for the help with laboratory work and creating a work atmosphere in the laboratory in which you always feel welcome and inspired.

To my friends, Isak Kvitfjell, Brage Riise, and my sister Therese Olsen for lightening up my weekdays.

To my parents for always believing in me. This would have been impossible without your lifelong support and love.

Mathias Eilert Olsen

Trondheim, May 15th, 2021

Abstract

The marked for fresh aquaculture fish is increasing as fish can provide several important nutrients to the human diet including essential omega-3 fatty acids. The use of fresh produce is however limited due to high susceptibility of microbial and biochemical spoilage. Earlier studies show that modified atmosphere packaging (MAP), antioxidant addition and temperature control can prolong the shelf life of fresh fish. The aim of the current study was to investigate the combination of natural antioxidant, MAP and temperature control in order to prolong the shelf life of Atlantic salmon.

The study was conducted in two storage experiments lasting 16 days each. The first storage experiment combined MAP (CO_2:N_2 60:40) and vacuum-packed samples with cold storage at 4°C and 0°C. The second combined MAP (CO_2:N_2 60:40) at 0°C with treatment of rosemary extract (0,5% and 0,05 w/v) and tocopherols (0,5% and 0,05% w/v) in ethanol. Quality changes during storage were analysed in terms of drip loss, microbial growth, and measurement of primary and secondary oxidation products by PV and TBARS.

Vacuum packed samples form the first storage experiment had a higher drip loss than samples stored in MAP. No significant difference in drip loss between samples treated with antioxidants compared to the control in the second storage experiment was found. Samples stored at 4°C had a higher microbial growth than samples stored at 0°C regardless of packaging method in the first experiment. There was no significant difference in microbial growth between antioxidant treated samples and the control in the second storage experiment. There was a significant difference between PV values from day 1 and day 16 for both the first and second storage experiment, but no significant difference between treatment and/or storage was found in either. There were no significant differences in TBARS values between day 1 and day 16, or between storage and/or treatment between any samples from either the first or the second storage experiment.

In conclusion, this study showed the effect of temperature in terms of microbial growth on Atlantic salmon, and the effect of MAP in retaining the quality. The study was unable to detect any effect of antioxidant treatment to lipid oxidation, microbial growth or drip loss.

Sammendrag

Markedet for fersk havbruksfisk er økende ettersom fisk kan være en kilde for viktige næringsstoffer i den menneskelige dietten, som omega-3 fettsyrer. Bruken av ferske råvarer er derimot begrenset grunnet høy sensitivitet for mikrobiell og biokjemisk bedervelse. Tidligere studier viser at modifisert atmosfærepakning (MAP), antioksidanter og temperatur kontroll kan forlenge holdbarheten for fersk fisk. Målet med denne studien var å undersøke kombinasjonen av naturlige antioksidanter, MAP og temperaturkontroll for å forlenge holdbarheten for atlantisk laks.

Studien ble gjennomført i to lagringsforsøk som varte i 16 dager hver. Det første forsøket kombinerte MAP (CO_2:N_2 60:40) og vakuumpakkede prøver med kjøling på 4°C og 0°C. Det andre lagringsforsøket kombinerte MAP (CO_2:N_2 60:40) ved 0°C med antioksidant behandling av rosmarinekstrakt (0,5% og 0,05% vekt/volum) og tokoferoler (0,5% og 0,05% vekt/volum) i etanol. Endringer i kvalitet under lagring ble analysert gjennom drypptap, mikrobiell vekst og målinger av primære og sekundære oksidasjon produkter i from av PV og TBARS.

Vakuumpakkede prøver fra det første lagringsforsøket hadde høyre drypptap enn prøver lagret i MAP. Ingen signifikant forskjell i drypptap mellom prøver behandlet med antioksidanter sammenlignet med kontrollen ble funnet i det andre forsøket. Prøver lagret ved 4°C i det første lagringsforsøket hadde høyere mikrobiell vekst enn prøver lagret ved 0°C uavhengig av pakkemetode. Det var ingen signifikant forskjell i mikrobiell vekst mellom antioksidant behandlede prøver og kontroll i det andre lagringsforsøket. Det var en signifikant forskjell i PV mellom dag 1 og dag 16 for både det første og det andre lagringsforsøket, men ingen signifikant forskjell mellom behandling og/eller lagrings metoder for enten det første eller det andre lagringsforsøket ble funnet. Det var ingen signifikant forskjell mellom TBARS verdier mellom dag 1 og dag 16, eller mellom lagrings og/eller behandlingsmetode for noen av prøvene i det første eller det andre lagringsforsøket.

Til oppsummering viste denne studien effekten av temperatur på mikrobiell vekst i atlantisk laks, og effekten av MAP for å bevare kvaliteten. Studien klarte ikke å oppdage noen effekt med antioksidant behandling på lipid oksidasjon, mikrobiell vekts eller drypptap.

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Abbreviations

PUFA	Polyunsaturated fatty acid
MAP	Modified atmosphere packaging
PV	Peroxide value
TBARS	Thiobarbituric acid reactive substances
SSO	Specific spoilage organism
FRS	Free radical scavenger
TMAO	Trimethylamine oxide
TMA	Trimethylamine
CFU	Colony forming bacteria

1 Introduction

1.1 Background

With an increasing world population, the demand for sustainable and health foods, also increases. Food from marine sources may be an important key in this as they can be rich in protein, vitamin D and omega-3 fatty acids. Fish have a lower conversion rate that land animals, meaning more protein can be produces using less feed, which ultimately will save anergy and water usage. Further, fish consumption is promoted as part of a healthy diet containing nutrients which may not be obtainable from land sources, like vitamin D, and has been linked with a reduced risk of heart disease.

As aquaculture increases in production and economic value has increased the products also has to stay fresh under transportation to a wider marked. Also, due to environmental reasons in order to reduce food waste, the shelf life of the products is also factor then needs be prolonged. This can be challenging for fish, as they are highly perishable. This is especially true for fish from northern latitudes where the natural environment is colder, giving cold storage less of an effect (1).

As spoilage of fish is attributed mostly to microbiological spoilage and lipid oxidation, methods on which limits these, while still keeping the produce fresh needs to be tested. Previous work with vacuum and modified atmosphere packaging have proven to increase shelf life of fish yet retains strains of bacterium which can survive and multiply in the anaerobic conditions, the combination of modified atmosphere packaging with other preservative methods needs be tested.

1.2 Atlantic salmon (*Salmo Salar)*

Atlantic salmon (*Salmo Salar)* is a mostly anadromous species of the family Salmonidae found in the northern Atlantic Ocean and the rivers connected to it. Though it is usually anadromous, the species can be found in freshwater lakes completing their life cycles entirely within fresh water. The species' reproduction and nursery phases occur in freshwater before migrating to marine environments where they will experience a period of rapid growth before sexual maturity. Unlike a lot of pacific species of Salmonidae, Atlantic salmon does not die after reproduction and can therefore grow large in size, over 30 kg, usually found in Russian and Norwegian rivers (2).

Historically it has been found in Europe from Iceland to the Barents and Kara sea, and south to northern Portugal with the inclusion of the Baltic sea (3). However, wild stocks of the Rhine and Elbe, the many rivers that drain into the Baltic sea, as well as rivers in France, Spain and Portugal are no longer to be found, making the species range fragmented (2). This is largely attributed to industrialisation via the building of dams, pollution and dewatering (4).

Production of farmed salmon has been ever increasing since the 1970's, with Norway being one of the largest exporters of farmed Atlantic salmon in the world. Nowadays farmed Atlantic salmon far surpasses wild salmon fisheries production.

1.3 Composition of fish muscle

Within fish there is a very clear distinction between white meat, which constitutes the bulk of the edible part of the fish, and red or dark muscle. In white fish such as cod and haddock the dark muscle can be found as a thin layer under the skin, concentrated in lateral lines (5). Fatty fish have a larger proportion of dark muscle which is also rich in fats (5). The lipid content of fish muscle varies greatly between species, where non-fatty fish will have an average lipid content of about 0,5% while fatty fish will have lipid contents ranging from 3-25% (1). Otherwise, fish muscle contains about 3% connective tissue, 15-20% protein, 1% and carbohydrates (1).

1.4 Microbial spoilage of fish meats

Generally, the meats of fish, as well as their organs, are usually sterile. Contamination can come from exposure to air, handling or from gutting, or from exposure to gut bacteria. Within the gills and the gut, the number of colony forming bacteria (CFU) usually ranges between 10^3-10^9 per gram (1) (6). These bacteria are mainly Gram-positive bacteria such as *Pseudomonas*, *Shewanella*, *Psychrobacter*, *Vibrio*, *Flavobacterium* and *Cytophaga*. The microflora is also dependent on the temperatures of their habitat which can cause fish from northern temperatures to not last as long in ice than fish from temperate waters. Bacteria found in fish from salt waters usually have a range of salt concentrations in which they can grow, while not strictly being halophilic (1).

Due to the low content of carbohydrates post-mortem acidifications is usually limited in fish, thereby making it more susceptible to bacterial growth. Further, with carbohydrates not

available to microbes the bacteria will immediately resort to using assimilated nitrogenous compounds. An example is trimethylamine oxide (TMAO) found in significant quantities in marine fish as part of the osmoregulatory system (1). This can be used as a terminal electron acceptor by non-fermentative bacteria such as *Shewanella putrefaciens*. The product of said reduction is trimethylamine (TMA), which is a core component in the characteristic odour of fish (6). The combination of near-neutral pH from limited post-mortem acidification and the availability of TMAO can lead the previous mentioned *Shewanella putrefaciens* to produce TMA and hydrogen sulfide causing spoilage (1) (6).

Though the micro biome of fish may be diverse, usually the spoilage process is the result of the specific spoilage organisms (SSO). *S. putrefaciens* is an SSO under both aerobic conditions, and under anaerobic conditions in MAP packaging together with *Lactobacillus*, and *Photobacterium putrefaciens* (6).

1.5 Chill storage

Most chemical reactions are temperature dependent. As food spoilage is usually a result of chemical reactions mediated by microbial and endogenous enzymes a lowering of temperature should increase the shelf-life of most foods. Chill storage, storing food slight above their freezing point (0-5°C), can not only change the rate at which spoilage happens, but also the nature of it. Lower temperatures cause selective growth of microflora, often favouring psycotrophs. Though they can grow in chilled foods, they do so slowly, and thereby small temperature changes within chilled temperatures can have pronounced effects (1).

The ability of organisms to grow at low temperatures is associated with the composition of the plasma membrane. At lower temperatures plasma membranes will transform to a rigid gel in which transport across it is severely limited. The temperature in which this happens for psychotrophs is lower as their plasma membrane consists of more unsaturated and short fatty acid chains. This phase change can cause death and injury via cold shock, but will most likely not kill all mesophiles, and mesophiles still have the ability to recover from injuries and reproduce after the phase change (1).

1.6 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is a pack flushed through with a gas mixture, usually of carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2). The gas combination in MAP will change during storage due to microbial respiration, dissolution of CO_2 in water, and gas exchange across the packing membrane. The gas combination is chosen to not affect the storage condition of the product in a negative way. CO_2 is often chosen for its inhibitory effect, N_2 to retain pressure and reduce a collapse of the packaging as it has low water solubility, while oxygen is used to retain colour in red meats (1).

Four ways on how CO_2 can influence and upset the normal physiological equilibrium has been found: alteration of cell membrane function, inhibition of enzymes or decrease the rate of enzymatic reactions, intercellular changes in pH, changes in pysico-chemical properties of proteins (6). Lower temperatures will increase the solubility of CO_2 and can thereby also increase the effect MAP packaging.

1.7 Lipids

Lipids are in general a heterogenous group of non-polar substances associated with living systems. This includes dietary oils and fats, phospholipids of cell-membranes and steroids. For food chemists and within food production fatty acids are lipids of higher interests. Though fatty acids are an important source of energy, and can be found in a variety of foodstuff (5) it is usually accepted that polyunsaturated fatty acids (PUFA) of an greater importance of consumption rather than saturated fatty acids due to potential health benefits. Humans synthesise fatty acid chains no longer than 9-10 carbons long, meaning PUFA needs be provided via dietary means (5).

1.8 Lipid oxidation

An example of lipid oxidation is oxidative rancidity, a form of lipid peroxidation, which can affect oils, fat and the fatty part of meat and fish and result in autooxidation of unsaturated fatty acids. The reaction causing autooxidation is divided in three parts: initiation, propagation, and termination (5) (7). The outline of an example pathway of the reaction is shown in figure 1.1.

During initiation, the abstraction of a hydrogen from a fatty acid forms a fatty acid radical known as the alkyl radical. This free radical stabilises by delocalisation over the double bonds resulting in the double bonds shifting, or, for PUFAs, the formation of conjugated double bonds. This can produce double bonds in either the *cis* or *trans* configuration, though *trans* is usually favoured due to its greater stability. The ease of forming said fatty acid radicals increase with an increasing unsaturation. In PUFAs the double bonds are in a pentadiene configuration with a methylene-interrupted carbon. The covalent bond between hydrogen and carbon in this configuration is significantly weaker than hydrogen-carbon bonds in aliphatic systems meaning it is easier for hydrogen abstraction to take place at these sites. And increase in unsaturation will increase the number of these methylene-interrupted carbons, cause a doubling of oxidation with the addition of a methylene-interrupted carbon (7).

During propagation, atmospheric or triplet oxygen will be added to the alkyl radical. The free radicals in triplet oxygen are usually too low energy to initiate hydrogen abstraction by themselves. The formation of peroxyl radical with the addition of oxygen to the alkyl radical will result in a high energy radical capable of hydrogen abstraction from a carbon-hydrogen covalent bond in an unsaturated fatty acid. This will cause the formation of a fatty acid hydroperoxide and the formation of a new alkyl radical thus propagating the reaction from one fatty acid to another (7).

During termination, two radicals will combine to form non-radical species. In the presence of oxygen, the predominate radical will be the peroxyl radical. Therefore, under atmospheric condition termination reactions may occur between peroxyl and alkoxyl radicals. In low oxygen environments termination reactions can occur between alkyl radicals to form fatty acid dimers (7).

The initial free radical can be created via a reaction with a singlet oxygen ($^1O_2$) created from a ground state triplet oxygen ($^3O_2$) in a reaction scheme involving pigments such as chlorophyll, riboflavin and haem, and light (5).

The kinetics of lipid oxidation in foods often have a lag phase follow by an exponential increase in oxidation rate. For foodstuffs, the length of the lag phase is important due to rancidity not being detected within this period. Once the exponential phase is reached lipid oxidation proceeds quickly and off-putting aromas will develop rapidly. However, lipid autooxidation reaction does not increase the net number of free radicals and therefore cannot be the only mechanism in which lipid oxidation happens. This can be attributed to the

presence of prooxidants which can accelerate lipid oxidation by direct interaction with unsaturated fatty acids to form lipid peroxides, or via promoting formation of free radicals. Hydroperoxides do themselves, though, not contribute to off aromas in food and do not directly cause rancidity (7).

Hydroperoxides will after a while decompose into alkoxyl radicals in which a myriad of reaction schemes can occur based upon the fatty acid type as well as the location of the hydroperoxide in the fatty acid. Further, decomposition products may be unsaturated themselves and have intact pentadiene structures meaning the oxidation products can be further oxidised. Since the fatty acid composition varies greatly in food, the decomposition products will vary greatly as well. As alkoxyl radicals are more energic than either alkyl or peroxyl radicals they are able to abstract electrons from the covalent bonds adjacent to the alkoxyl radical causing cleavage of the aliphatic chain of fatty acids decomposing fatty acids low molecular weight compounds which will be perceived as rancidity (7).

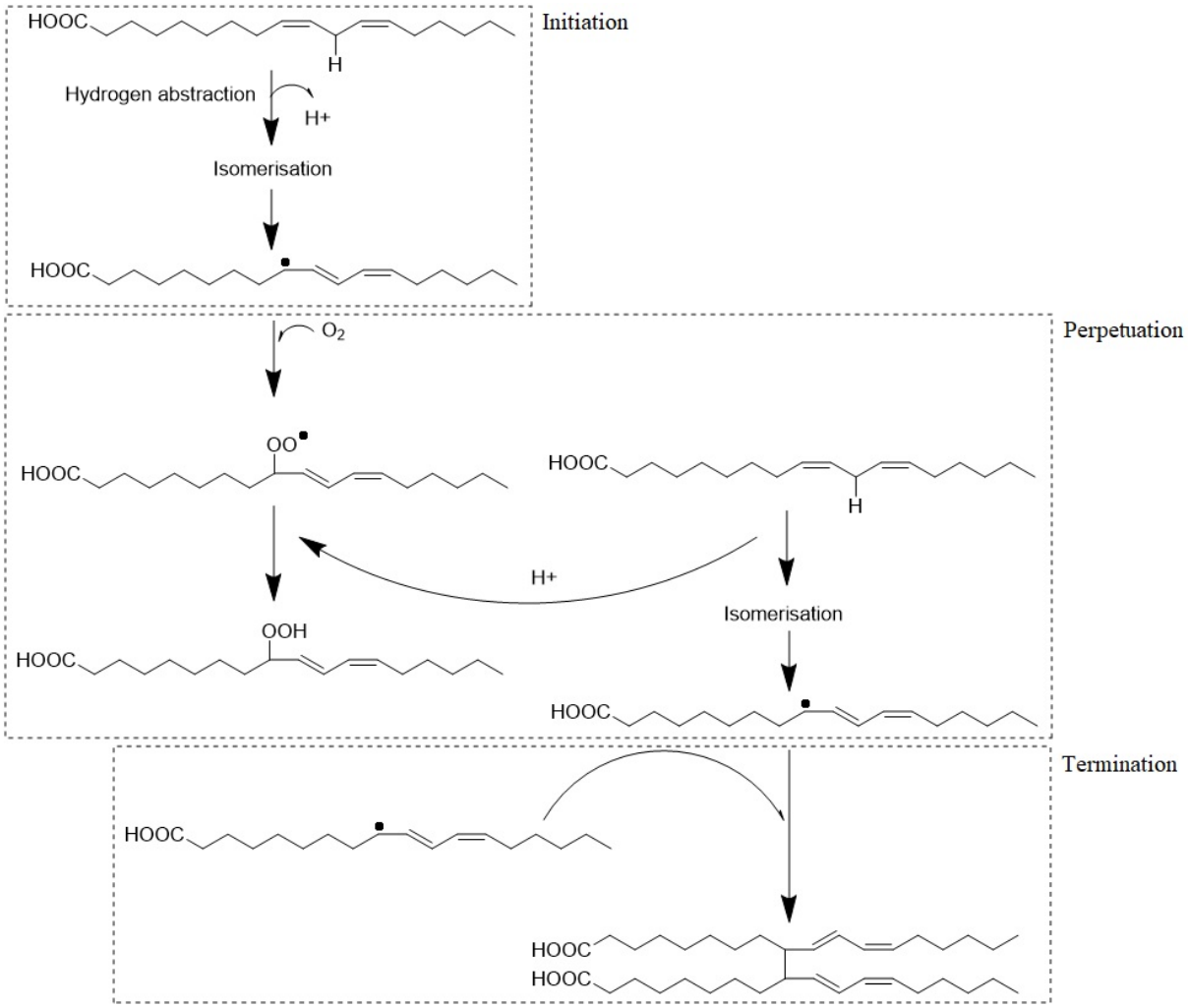

Figure 1.1: General scheme of one pathway of lipid autooxidation in linoleic acid (7).

1.9 Antioxidants

Antioxidants are molecules capable of slowing down or preventing oxidation from other molecules. Oxidation reactions can generate toxic metabolites, including free radicals, which can start chain reactions damaging tissues and cells. Antioxidants would terminate these chain reactions by removing free radical intermediates and derivatives and thereby inhibit further oxidation reactions by themselves being oxidised (7, 8). Though some oxidation may be beneficial others can lead to degradation of lipids, vitamins, nutrient, pigments and lead to development of off-flavours (7). Antioxidants can be found in fruits, vegetables, nuts, grains and some meat, poultry, and fish. Examples of naturally occurring antioxidants include vitamin C and E, though synthetic antioxidants also exist including butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Synthetic antioxidants are common additives to lard to prevent rancidity and prolong the shelf-life of pastry where the fatty components are more exposed and thereby more susceptible to oxidation. However, there are health concerns regarding synthetic antioxidant, among them being carcinogenic (5, 9). Synthetic antioxidants are, though, cheaper to produce, often have higher antioxidant activity than natural occurring antioxidants, and are often less polar meaning they can more easily be solved in lipids (9). There is therefore a growing interest in the use of organic and naturally occurring antioxidants.

Antioxidant activity can come in two form: as primary or secondary antioxidants. Primary antioxidants work by neutralising free radicals by donating a hydrogen atom or via single electron transfer. Secondary antioxidants on the other hand neutralise peroxide catalyst such as iron and cobber, or by deactivating reactive species such as $^1O_2$. BHA and BHT are examples of primary antioxidants whereas ethylenediaminetetraacetic acid (EDTA), citric acid and beta-carotene are examples secondary antioxidants (10).

Among primary antioxidants there are free radical scavengers (FRS) which inhibit lipid oxidation by interacting faster with free radicals than unsaturated fatty acids. This is mainly done via the peroxyl radicals. The antioxidant efficiency is dependent on the ability of the FRS to donate hydrogen to a free radical. Phenolic compounds possess many of the properties required from an efficient FRS, donating hydrogen from their hydroxyl group and delocalise the formed radical via its phenolic ring structure. Often its effectiveness is increased by substitution groups on the phenolic ring that increase the ability to donate hydrogen to lipid radicals or increase the stability of FRS radical (7).

1.10 Rosemary

Plants contain a diverse group of phenolic compounds, all which meet the structural requirements of FRS, although their activity vary widely. Among the most commercially important source of natural phenolics is rosemary extracts.

Rosemary (*Salvia Rosmarinus*) is a shrub native to the Mediterranean region and a member of the mint family Lamiaceae. The reason for Rosemary's antioxidant properties is due to it containing a broad range of phenolic compounds including carnosic acid, carnosol and rosmarinic acid shown in figure 2 (11). The components vary in their hydro- and lipidophilic qualities, distributing them along a spectrum of solubility and polarity mean they can act as power antioxidant species in polar and non-polar phases in more complex food (12).

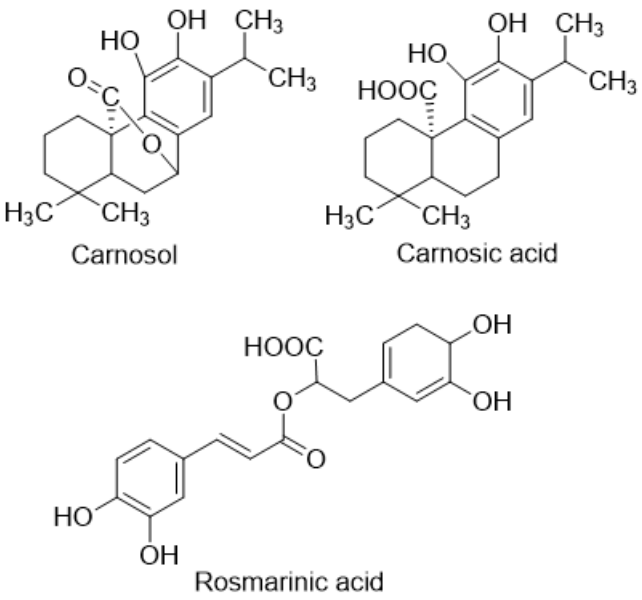

Figure 1.2: The structural formula of three phenolic compounds found in rosemary extracts: carnosol, carnosic acid and rosmarinic acid (7).

1.11 Tocopherols

Tocopherols are viscous oils where lower molecular weight members have melting points below 100°C and are found naturally occurring in a variety of plant species, especially within plant oil (13, 14). They are generally associated with vitamin E in which four tocopherols are part of said group: α-, β-, γ- and δ-tocopherol. These differ in the position of one or more methyl groups at 5', 7' or 8' position on the ring structure present in tocopherols, which further also affects the vitamin E activity of the tocopherol (7, 13). The general structural formula for tocopherols is shown in figure 3. The α form of tocopherol is usually seen as the sole form exhibiting specific vitamin E activity and is also the form which is transported easiest within the human body (13, 15, 16). The most well-known function of vitamin E is that of a chain breaking antioxidant that prevents cyclic propagation of lipid peroxidation (5, 16). This process helps prevent the onset of rancidity in the oil, but peroxide build-up within the oil will after a while cause oxidation of the tocopherols to the point of lacking vitamin E activity (5).

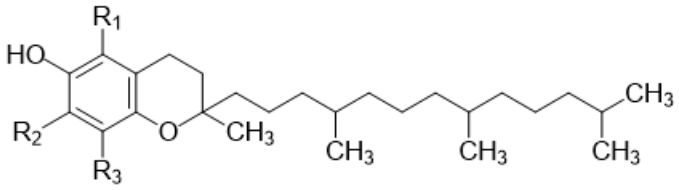

Figure 1.3: General structure for tocopherols. For α-tocopherol R_1, R_2 and R_3 are methyl groups. For β-tocopherol R_1 and R_3 are methyl groups. For γ-tocopherol R_2 and R_3 are methyl groups. For δ-tocopherol only R_3 is a methyl group (5, 7).

1.12 Determination of primary and secondary oxidation products

As previously mentioned, the oxidation of fatty acids can result in formation of oxidation products. The mere complexity of the pathways within lipid oxidation can be a challenge, though via analytical methods the presence and amount of certain oxidation products can be determined. This can either be primary or secondary oxidation products, both of which has their advantages and flaws in determining the rancidity of an oil or fat.

Primary oxidation products are the products created from initiation and propagation of lipid oxidation. Since they are the first oxidation products, they can appear early in the oxidation process of lipids. However, during the latter stages of lipid oxidation the rate in which the primary oxidation products decomposes increases compared to their formation, the concentration of these products will decrease. Further, the primary oxidation products are not volatile, and though there can be large amounts of them in a product, it would still not corelate with rancidity. This can produce misleading results as very rancid oils could have very low concentrations of primary oxidation products (7).

Secondary oxidation products are compounds produced from the decomposition of hydroperoxides. These compounds can generate a myriad of compounds both volatile and non-volatile. It can therefore be quite difficult to measure all these compounds at the same time so most analytical methods focuses on a single compound or a class of compounds. The drawback of measuring secondary oxidation products is that they are derived from primary oxidation products and thereby the concentration of secondary oxidation products can remain low while primary oxidation products remain high. However, these methods directly measure the compounds that are associated with rancidity, and thereby has a higher correlation with sensory analysis.

A way of measuring primary oxidation products is to measure fatty acid hydroperoxides. This is usually done via the hydroperoxides ability to oxidise an indicator compound. The most common way is the use of hydroperoxide-promoted conversion of iodide to iodine in a titration with sodium thiosulfate (NaS_2O_3). Peroxide values (PV) are via this method express as milliequivalents (mEq) of oxygen per kg of oil with 1 mEq equal to 2 mmol of hydroperoxide.

A way to measure secondary oxidation products is via thiobarbituric acid's (TBA) reaction with carbonyls. The compound most often attributed to be detected by TBA is malondialdehyde (MDA) whose TBA adduct absorbs strongly at 532 nm. MDA is produced in a two-step oxidative degradation of fatty acids with three or more double bonds. This means that the amount of MDA is dependent on the composition of the fatty acid being oxidised, and therefore TBA reactive substances (TBARS) should be measured for fresh produce before measuring oxidised produce in order to minimise interference from TBA reactive substances that are not products of lipid oxidation.

1.13 Application of fat-soluble antioxidants

For applying antioxidants to filets or other meat products it is common apply them to the surface area of the produce usually via applying water diluted antioxidants via spray or submersion in a solution. However, tocopherols and a large amount of the components of rosemary extracts remain hydrophobic meaning the methods used to apply water soluble antioxidants to meat needs be modified.

Previous studies on rainbow trout and salmon has used water dispersions of rosemary extracts, some containing as high concentrations as 10% w/w rosemary in water (17) (18) (19). In these studies, rosemary extracts are dissolved in water with specific concentrations in which fillets from rainbow trout have been submerged into in order to apply the antioxidant to the meat.

Though unlike rosemary, tocopherols remain close to insoluble in water, meaning that water dispersions cannot be used for both antioxidants. However, tocopherols are soluble in water/ethanol mixtures, where the more ethanol in the mixture the more tocopherols are soluble (15). This gives a new challenge however as microorganism are ill suited to handle most organic solvents (20) (21).

Several methods to use antioxidants to prolong shelf life of fish meats has been used including as an active film (22) (23) (24) (25) protein coating (26) or via dietary means.

1.14 Aim of thesis

The aim of the study was to investigate the effect of temperature control in combination with MAP and vacuum, and further test if, whatever combination of temperature and packaging methods works best, the shelf life and quality in Atlantic salmon could be further prolonged with the addition of tocopherols and rosemary, and the effect of concentration. To address this the study was conducted in two parts, the first with vacuum-packed and MAP samples stored at 0°C with controls stored at 4°C. In the second experiment, the temperature and packaging method that worked best, was tested with the addition of antioxidants at two different concentrations.

2 Materials and methods

This study was done in two separate storage experiments, the first (batch 1) lasting from 20.10.21 – 05.11.20 and the second one (batch 2) lasting from 09.03.21 – 25.03.21. The first experiment was done storing MAP and vacuum- packed samples at 0°C and 4°C for 16 days. The second was done using only MAP at 0°C, but with the application of rosemary extracts and tocopherols as antioxidants.

2.1 Equipment

Equipment	**Details**
Tray sealing machine	Webomatic TL250
Vacuum chamber machine	Webomatic supermax
Headspace gas Analyser	PBI Dansensor CheckMate 9900
Cold room	4°C
Digital scale	.01 g accuracy range
Digital scale	.0001 g accuracy range
Stomacher bag	
Stomacher homogeniser	
Vortexer	MS2 Minishaker IKA
Filter plates	CompactDry TC
Incubator	
Homogeniser	Ultra Turrax T25
Centrifuge	Multifuge X1R, 20°C
Centrifuge	Sorvall RC-5B Plus
Spectrophotometer	Ultrospec 2000
Klimax glass tubes	
Evaporating unit with N2-gass	Pierce Reacti-Vap
Heating module	Reacti-Therm
Titration beaker	
Aluminium foil	
Magnetic stirrer	TM235
Automatic titrator	TitroLine 7000 with platinum electrode Pt 62/61

2.2 Chemicals

Chemical	Details
Rosemary solution	0,5% and 0,05% w/v ethanol
Tocopherol solution	0,5% and 0,05% w/v ethanol
Ethanol (CH_2H_5OH)	
Methanol (CH_2OH)	
Carbon dioxide (CO_2)	
Nitrogen gas	
Saline peptone water	0,1% peptone, 0,85% NaCl
Chloroform	$CHCl_3$
Acetic acid (CH_3COOH)	Glacial
Potassium iodide (KI)	4,63 M
Sodium thiosulphate ($Na_2S_2O_3$)	0,001 M
2-thiobarbituric acid (TBA)	0,04 M
Sodium sulphite (Na_2SO_3)	0,3 M
Trichloroacetic acid (TCA)	0,28 M
1,1,3,3-tetraethoxypropane (TEP)	0,1 mM
Butylated Hydroxytoluene (BHT)	3% in ethanol

2.3 Natural salmon flora bacterial suspension preparation

In order to ensure surface bacteria on salmon treated with antioxidants in ethanol in batch 2, a salmon natural flora bacterial suspension was made on day 0 from the same salmon that batch 2 was made from. This was done by homogenisation of salmon meat in a stomacher bag filled with saline peptone water (0,1% peptone, 0,85% NaCl) and diluted to 2000 mL with saline peptone water (0,1% peptone, 0,85% NaCl). The CFU for this natural flora bacterial suspension was at $2{,}85*10^4$ per mL media.

2.4 Antioxidant solution preparation and application

The antioxidant extracts solutions used for batch 2 (rosemary and tocopherols) were prepared 08.03.21 by dissolving rosemary and tocopherols (0,25 g and 2,5 g) in ethanol (500 mL, 96%). The rosemary extract was obtained from Danisco, and the tocopherol mixture was obtained from DSM Nutritional Products Europe. They arrived in September 2020 and stored in a cold room (4°C) until the day of the antioxidant solution preparation. The solutions were further stored in a cold room (4°C) in glass bottles (500 mL) until the day of application.

The antioxidants were applied to the salmon by submerging each pre-cut piece in its assigned antioxidant solution for 4 ± 1 min and air dried before submersion in the natural flora bacterial suspension for 15 ± 5 min.

2.5 Packaging and storage

Atlantic salmon filets arrived in two batches at Kalvskinnet (NTNU) on October the 19th 2020 and March 8th 2021. Each batch was skinned and cut into equal sizes (50 ± 5 g, N=50 batch 1, 45 ± 5 g N=70 batch 2) the day after arrival. This was defined as day 0 of the experiment. The lean tail region was trimmed as well as areas of excess fat in order to keep the fat content as uniform as possible.

Batch 1 was divided into 4 treatment groups: vacuum 4°C (N=16), vacuum 0°C (N=16), MAP 4°C (N=16), MAP 0°C (N=16). Batch 2 was divided into 5 treatment groups: 0% antioxidant (N=16), 0,05% rosemary (N=16), 0,5% rosemary (N=16), 0,05% tocopherols (N=16), 0,5% tocopherols (N=16). In sum 50 samples were packed from batch 1 and 70 from batch 2. MAP treatment was carried out using a tray sealing machine (Webomatic TL250, 60:40 CO_2:N_2). The gas composition was analysed with a headspace gas analyser (PBI Dansensor CheckMate 9900) for three blank trays packed continuous with the samples. The average amount of CO_2 in the gas composition was determined found to be 60,9 ± 0,9% for batch 1 and 60,3 ± 0,2% for batch 2. Vacuum treatment was done using a vacuum chamber machine (Webomatic

Supermax). Batch 1 was packed as indicated on their treatment groups while all samples from batch 2 was MAP packed. After packaging the samples were transported to Gløshaugen (NTNU) and stored in a cold room (4 ± 1 °C) or in a refrigerator (0 ± 0,5 °C) for up to 16 days. Batch 1 was stored at the temperature indicated by their treatment group while all samples from batch 2 was stored in a refrigerator (0 ± 0,5 °C).

2.6 Experimental setup

The study was done in two parts, batch 1 and batch 2. After packaging and storage, they went through the same analyses.

Changes in microbial growth (total plate count), drip loss and lipid content were monitored after 1, 3, 6, 8, 10, 13 and 16 days of storage. After lipid extraction and determination of total lipid contents the chloroform extracts from each sample were stored in a freezer (-20°C) where a selection of samples was analysed for oxidation products (PV, TBARS). Figure 2.1 shows a flowchart of the experimental set up.

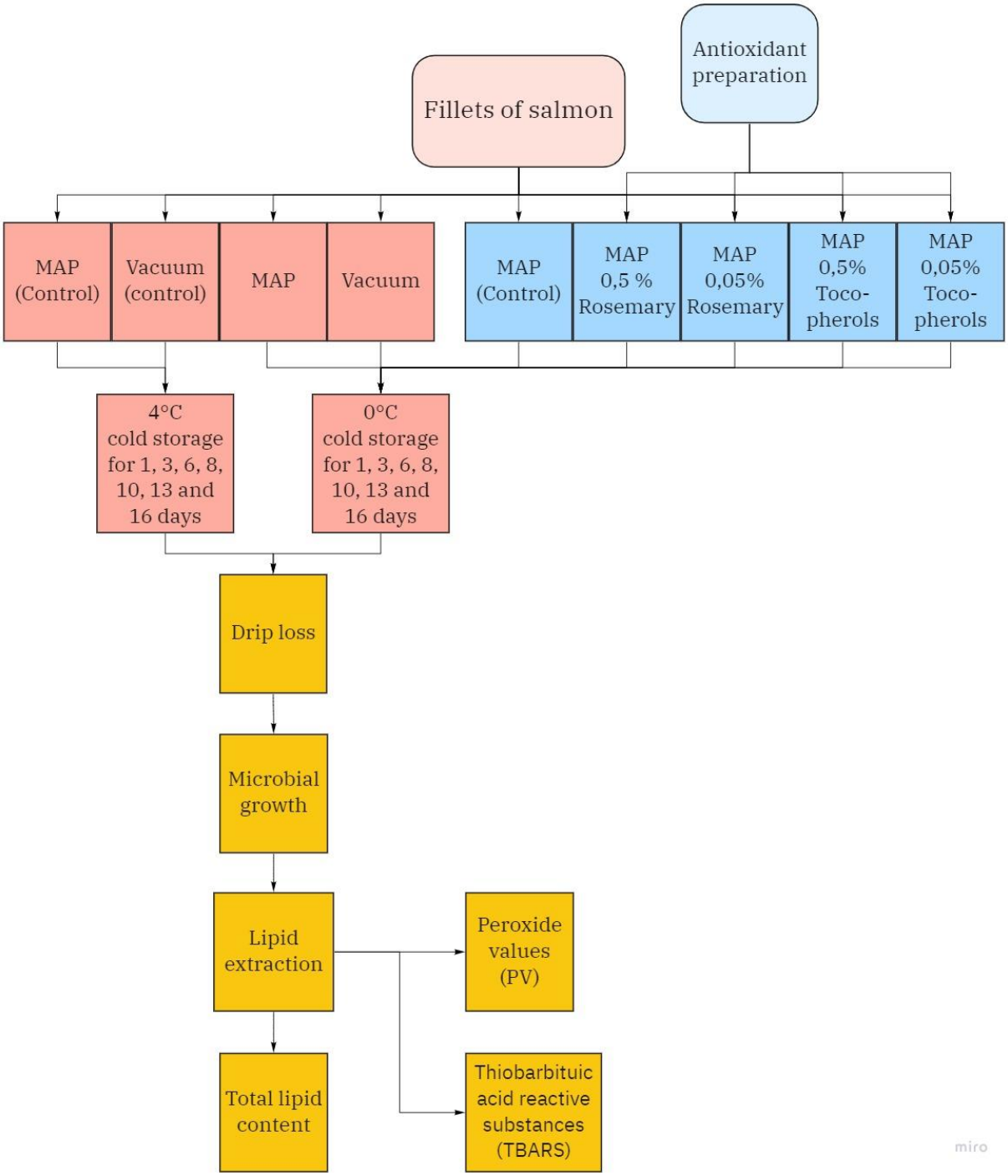

Figure 2.1: Flow chart for the experimental set-up.

2.7 Drip loss

The stored package was weighed on a digital scale (accuracy of 0.01 g). Excess drip in the bag (vacuum bag or MAP box) was removed using a paper towel for the fish and bag to be weighed separately once again. Equation 1 was used to calculate the drip loss in percent.

$$\text{Drip Loss (\%)} = \frac{\text{Mass of drip}}{\text{Mass of initial product}} * 100\% \quad (2.1)$$

Mass of drip was determined via the weight difference of the fish and the bag before and after removing excess drip. Mass of initial product was determined as weight of the stored package minus the weight of the dried box.

2.8 Microbial growth

10 ± 1 g samples were weighed out on a digital scale (accuracy of 0.01 g) and homogenised using a stomacher bag (Grade, 180 mm x 300 mm x 70 µm) in a stomacher homogeniser (30 sec, 230 rpm). A saline peptone water solution (100 mL, 0,1% w/v peptone, 0,85% w/v NaCl) was added to the stomacher bag before homogenisation. The suspension was used to create a dilution series of four with an increased degree of dilution to compensate for an increased microbial growth. 1 mL of diluted samples were plated out using filter plates (CompactDry TC) and incubated (30°C, 4 ± 1 days). Visible colonies were counted manually and mean values of CFU for each treatment were calculated as the average of countable plates from two parallels of the treatment. Microbial condition was reported as average CFU per g sample.

2.9 Lipid extraction

Chloroform extracts were made using the Bligh and Dyer method (27). Homogenisation was done using a homogeniser (Ultra Torrax T25).

2.10 Total lipid content

Kimax tubes were pre-weighed and added lipid containing chloroform extracts (1 mL). The chloroform was evaporated using a stream of N_2 gas from an evaporator unit (Pierce Reacti-Vap) and a heating module (Reacti-Therm, 60°C) for 20 ± 5 min. The tubes were then stored in a desiccator (>12 hours) and weighed.

For batch 1 the total lipid content was determined instantly as the chloroform extracts were made. For batch 2 total lipid content was determined instantly for all but samples from day 13 and day 16 which were stored in a freezer (-20°C) prior to total lipid content determination.

2.11 Primary and secondary oxidation products

As an indicator of primary oxidation products in the samples, the peroxide values (PV) were determined via iodometric titration of the chloroform extracts as described in AOCS official methods (28). PV was measured once for each treatment with three parallels for day 1 and day 16. If one of the parallels were outlies the value would be rejected and the two remaining values would be used to determine the average. In cases where none of the values corresponded new chloroform extracts would be used a parallel treatment group.

The titration was carried out using an automatic titrator (TitroLine 7000) coupled with a platinum electrode. For each titration day a fresh potassium iodide solution was made (KI) which was stored in a refrigerator (4°C) when not in use. At the end of the titration the volume of titrant consumed for the blank was noted down. The same procedure followed for titration of the samples replacing the chloroform with lipid containing chloroform extracts from the samples. PV was calculated using equation 2 and expressed as meq peroxide/kg lipids:

$$\mathrm{PV}\left(\frac{\mathrm{m_{eq}peroxide}}{\mathrm{kg\,lipid}}\right) = \frac{(\mathrm{V-B})*\mathrm{T}*1000}{\mathrm{w}} \quad (2.2)$$

V is the volume of titrant consumed (mL), B is the volume of titrant consumed for blank (mL), T is titrant (0,001 M), w is weight of lipids in sample.

Secondary oxidation products were determined by determination of Thiobarbituric acid reactive substances (TBARS). The method was modified from the micro method of Ke and Woyewoda (29). Modifications include the working solution containing 9,45 mL of 3 % BHT solution in ethanol in addition to TBA stock solution (180 mL), chloroform (120 mL) and sodium sulphite solution (15 mL). Kimax tubes were added 400 µL chloroform extract and 5 mL TBA working solution before boiling for 45 mins and cooling in cold water. TCA (2,5 mL) was added and the tubes were centrifuged (Multifuge X1R, 1000 g, 10 min, 20°C).The standard curve was made replacing chloroform extracts with diluted 1,1,3,3-tetraethoxypropane (TEP, 400 µL) with TEP amounts ranging from 0 – 15 nmol. These samples went through the same treatment as the chloroform extracts. After centrifugation, the samples were placed in a spectrophotometer (Ultrospec 2000, 538 nm) for measurement of absorbance. The analysis was performed in duplicate from one treatment method on samples from day 1 and 16.

The amount of secondary oxidation products in terms of TBARS were calculated using equation 2.3 and expressed as µmol TBARS per gram lipid.

$$TBARS\left(\frac{(\mu mol\,TBARS)}{g\ lipid}\right) = \frac{A-b}{a*w*1000} \quad (2.3)$$

Where A is the absorbance of sample, b is the intercept of the standard curve, a is the slope of the standard curve, w is the weight of lipids in the sample (g) and 1000 is the conversion factor from nmol to µmol.

2.12 Data processing and statistical analysis

Data management and analysis were performed using a spread sheet software (Microsoft Excel). Two- sided t-tests were done using the data analysis ToolPak and used to determine statistical significance using a confidence interval of 95% ($P<0,05$). Presentation of results is on the format average ± standard deviation of the mean (SDOM).

3 Results and discussion

This section shows the result from the experiments and analysis outlined in section 2. Raw data is found in appendix A with sample calculations.

3.1 Drip loss

A graph illustrating the change in drip loss for batch 1 is shown in figure 3.1. In general, the drip loss for MAP samples remained low, with only an increasement occurring at day 6. The average drip loss remained around 1% for MAP samples, which is not considered to be high (30). Previous studies have shown the drip loss to increase with storage time (31) (32) (33) (34), however this change was not significant for MAP packed filets in this study. The overall change in drip loss between vacuum-packed and MAP packed samples was significant, while change in drip loss was not significant with which temperature the samples were stored at. This is in compliance with previous studies showing that vacuum tends to give higher drip loss compared to MAP (35). The same tendencies have been found in Atlantic salmon as well (36) (37).

Drip los can be corelated with bacterial growth as drip loss can work as a growth medium for bacteria, so the higher CFU per gram sample could correlate to an increase in drip loss. This due to structural damage caused by bacteria (30). No correlation was, however, found between CFU/g and drip loss.

Drip from vacuum-packed samples at 4°C at the end of study was found to have yellow tan. The same was not found for vacuum-packed samples 0°C. However, no formal sensory analysis nor colour analysis was conducted on this topic, so any observational data remains speculation.

Drip loss was also monitored in the second experiment. A graph showing the change in drip loss over 1-16 days for batch 2 is shown in figure 3.2. Here there is no significant difference between either storage methods, only in between days. Therefore, the addition of antioxidants in this study cannot be said to increase nor decrease drip loss. The general drip loss remains significantly higher than drip loss for MAP samples from batch 1. This could be due to the samples being suspended in a water-based bacterial suspension, even though the samples were dried after. The general drip loss remains low to medium still.

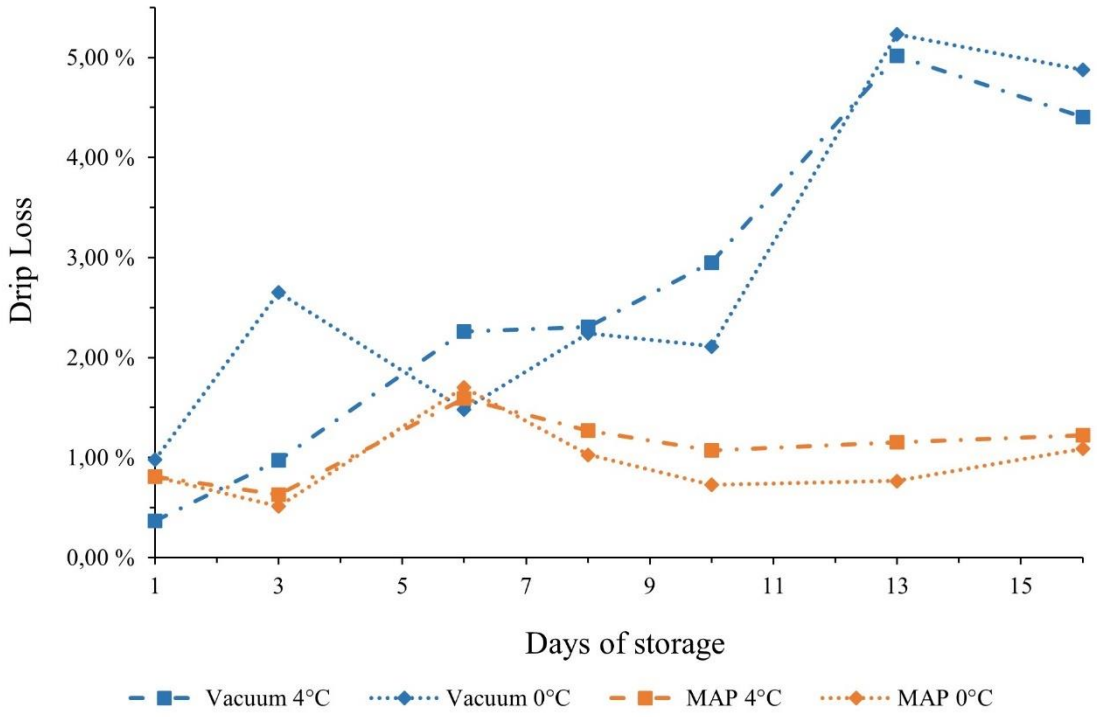

Figure 3.1: Drip loss (%) during 1-16 days of storage for vacuum at 4°C (blue, long stippled), vacuum at 0°C (blue, short stippled), MAP at 4°C (red, long stippled) and MAP 0°C (red, short stippled) samples.

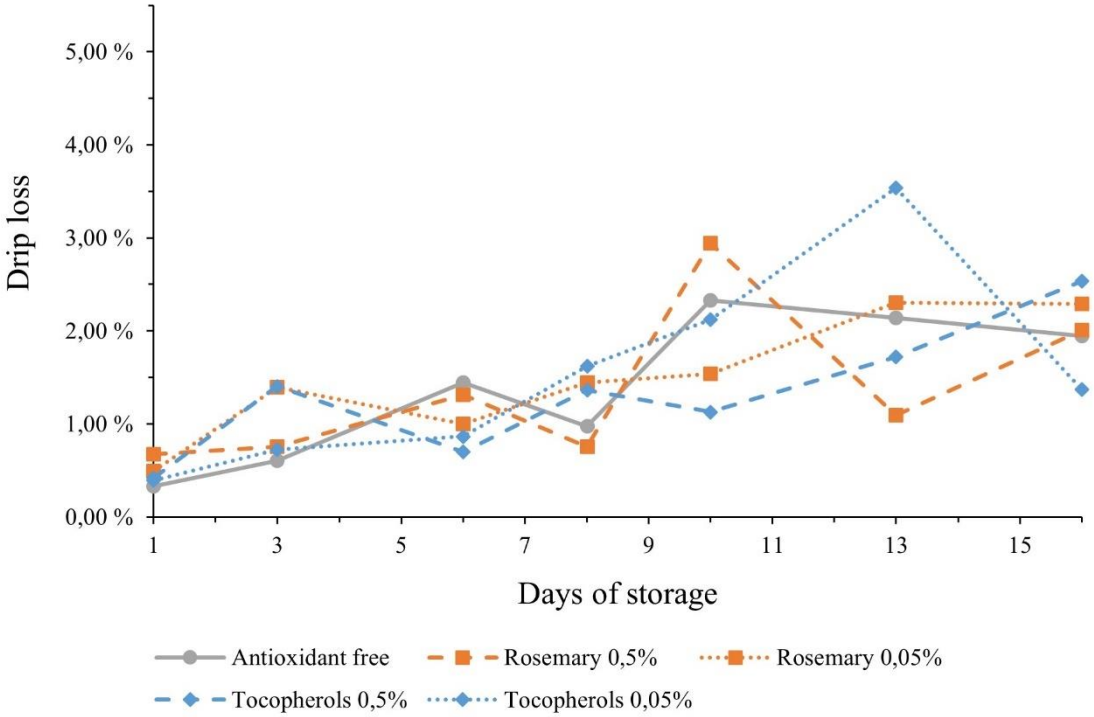

Figure 3.2: Drip loss (%) during 1-16 days of storage for different treatments: antioxidant free (grey), rosemary extract 0,5% (red long stippled) rosemary extract 0,05% (red short stippled), tocopherols 0,5% (blue long stippled) and tocopherols 0,05% (blue short stippled).

3.2 Microbial growth

The microbial growth for MAP and vacuum-packed salmon in batch 1 is shown in figure 3.3. There is a significant difference between samples stored at 4°C and 0°C ($P<0,05$). There was no significant difference between samples stored at 4°C regardless of packaging method ($P>0,05$). There seems to be a difference between vacuum-packed and MAP samples however. Previous studies have found a significant change in microbial growth between MAP and vacuum (36) (38) which may be in compliance with the small change seen between vacuum-packed and MAP samples stored at 0°C in batch 1. The total plate count was however low, as only low concentrations were plated out in the latter half of the experiment. for these samples meaning the CFU/g is highly inaccurate.

With microbial growth being so dependent on temperature it should be expected to see differences with temperature control. Under anaerobic conditions, however, which bacteria that works as SSO, or the rate at which they grown, is not affected by the atmosphere composition. Therefore, there should not be a significant difference between vacuum and MAP (1). This is due to *Photobacterium phosphoreum***,** a producer of TMAO and TMA being highly resistant to CO_2 where the extended shelf life of cod under anaerobic condition have been linked to the growth of *P. phosphoreum* (6). The same bacterium has been linked to spoilage of salmon (39) (36) (40).

According to the International Commission on Microbiological Specifications for Food (ICMSF), a general limit for acceptable CFU/g in fish for human consumption is at 10^6 (41). Vacuum-packed samples remained under CFU at 10^6 until day 13 while MAP packed samples remained under CFU 10^6 for the entirety of the study. Vacuum-packed samples at 4°C surpassed this threshold after day 6, while MAP-packed samples at the same temperature reached the threshold after day 8.

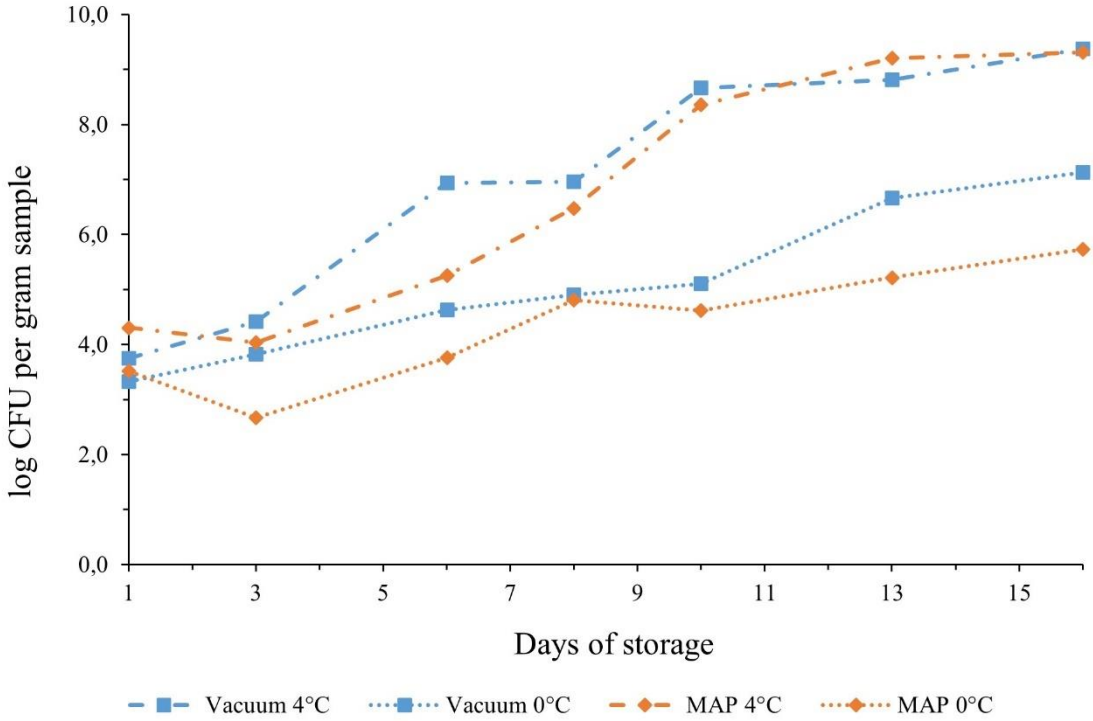

Figure 3.3: Colony forming bacteria (CFU) per gram salmon after 1-16 days of storage for samples stored in vacuum at 4°C (blue, long stippled), vacuum at 0°C (blue, short stippled), MAP at 4°C (red, long stippled) and MAP 0°C (red, short stippled) samples.

Microbial growth was also monitored for batch 2 was stored under the same conditions with no change in temperature nor change in packaging. As MAP packed samples at 0°C showed the best enhancement of storage time, this packing conditions were also chosen to be the one used with applied antioxidants.

The bacterial growth for antioxidant treated samples is shown in figure 3.4. At the end of the study there is so significant separation between the different treatments. In fact, the samples from day 1 have a higher CFU per gram sample than samples from day 16. This is probably due to contamination in the peptone water used, as the peptone water for day 1 and 2 was previously used for another storage study involving salmon samples. After these first two sampling days the peptone water was made and autoclaved within 1 day of sampling. This may be why the samples are more uniform in the days after.

The second explanation may be a surplus of bacteria after treatment from the natural flora suspension which die off during treatment. However, as they were stored for 1 day before any sampling was done, most of the bacteria unable to grow in the conditions should die off

quickly, but the samples do not start to converge until day 8. If this dip in CFU/g after day 3 was common to the treatment method, it should also be observed for MAP samples at 0°C from batch 1. There is a dip in CFU/g from day 1 to 3 for MAP samples, not observed in vacuum-packed samples from batch 1, however this dip is not statistically significant (P>0,05). Neither is the dip from day 1 and 3 for batch 2 statistically significant (P>0,05).

Regardless, most samples never surpass the 10^6 thresholds, except for tocopherols 0,05% on day 1. This may show that the treatment overall helps supress bacterial growth in the samples. However, in order to confirm that a control untreated with ethanol would have to be tested alongside treatment methods to confirm that. This study is however meant to look at the effect of antioxidants alone.

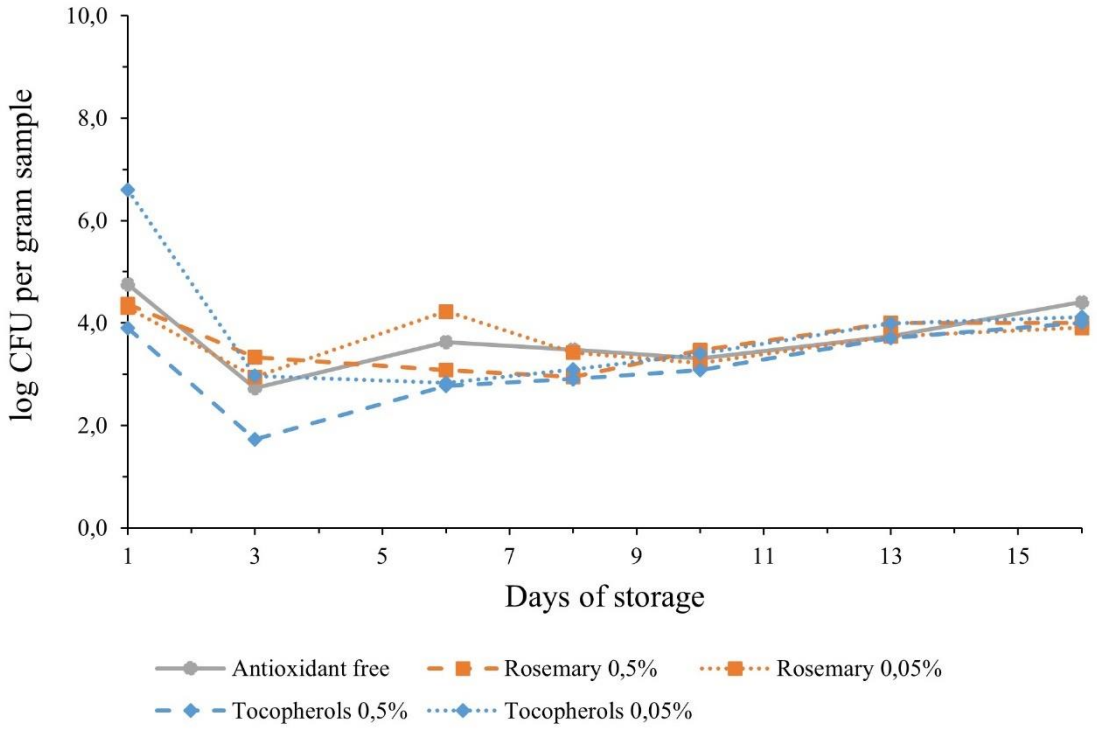

Figure 3.4: Colony forming bacteria (CFU) per gram salmon after 1-16 days of storage for samples for different treatments: antioxidant free (grey), rosemary extract 0,5% (red long stippled) rosemary extract 0,05% (red short stippled), tocopherols 0,5% (blue long stippled) and tocopherols 0,05% (blue short stippled).

The lack of divergency in CFU/g sample between antioxidant samples have also been found in previous studies in fatty fish (37) (42). In particular Mejlholm and Dalgaard (2002) (42) investigated differences between essential oil in lean and fatty fish where they suggested that the active components of the antioxidant dissolved in the lipid phase of salmon making them less available to act on bacteria in the aqueous phase. The application method used for the antioxidants in this study assumes the accumulation of antioxidants in fat in the fish so if this reduces any antimicrobial activities observed outside of storage experiments this may explain the lack of divergence between regular MAP samples and antioxidant treated samples. However, studies done on rainbow trout have shown a significant difference in trout filets treated with 0,5% rosemary EO compared to the control (19). However, Zinohartova et. al. (19) did only see the significant change between rosemary treated samples and the control after 8 days of storage when the CFU/g for all samples became higher than 10^5, having increased from 10^2 CFU/g. It could be the case that the samples from this study never reach growth period in which this separation could take place, as the CFU/g remains fairly low during the whole storage experiment.

3.3 Total lipid content

For the first batch of salmon the average lipid content for the samples was at 12,58%, the median at 11,73% with the lowest value at 2,90% and the highest at 24,05%. This is within expected values for lipid content of Atlantic salmon, especially if it is farmed as is the case in this study (37) (43) (44) (45). There was no significant difference between the lipid content of vacuum-packed and MAP packed samples. There was no significant difference between the lipid content of samples stored at 4°C and 0°C (P>0,05). The change in lipid content for each day and each treatment method for batch 1 is shown in figure 3.5.

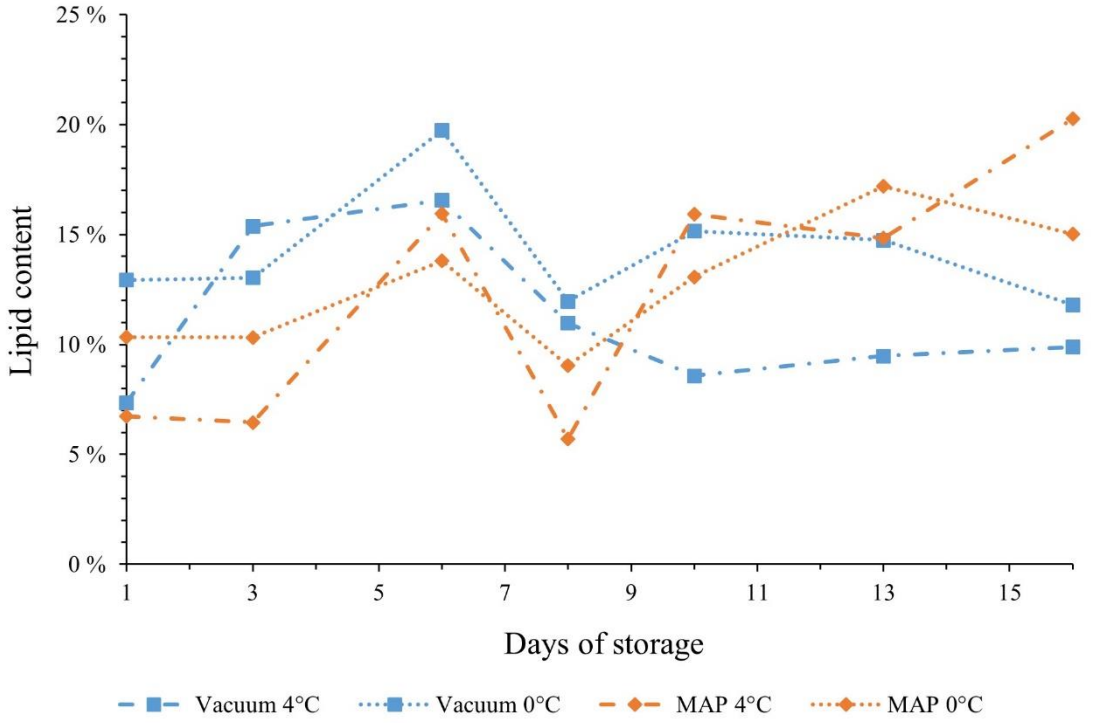

Figure 3.5: Lipid content (%) during 1-16 days of storage for vacuum at 4°C (blue, long stippled), vacuum at 0°C (blue, short stippled), MAP at 4°C (red, long stippled) and MAP 0°C (red, short stippled) samples.

The variation in lipid content is probably due to changes in individual differences in the salmon as well as changes in the lipid content within the filets themselves. In this study excess fat was trimmed off, however the samples came from different parts of the filets which could explain the rather large differences between lowest and highest values. It should be noted all samples even minimum and maximum values falls within lipid content changes that can be found in the same fillet (44).

The lipid content was also monitored for batch 2. The change in lipid content for each day for batch 2 is shown in figure 3.6. The average lipid content was 14,67% with the highest value being 24,19%, the lowest being 4,17% and the median being 14,46%. This is slightly higher than for batch 2 but still within expected lipid content to for farmed salmon (43) (44).

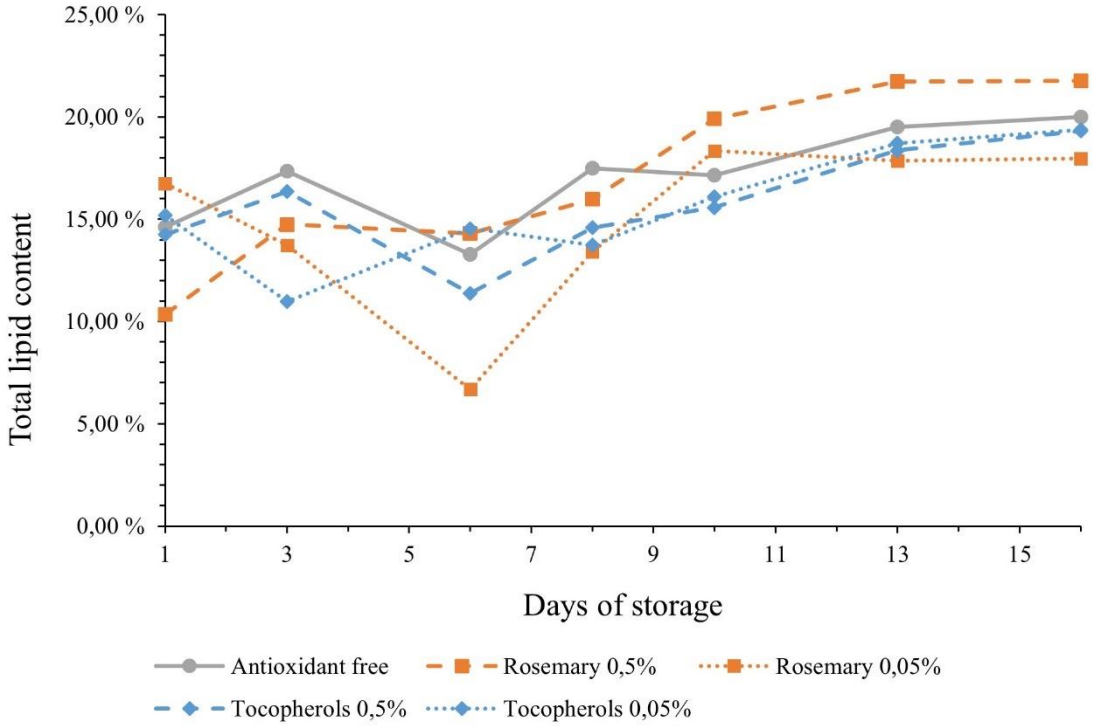

Figure 3.6: Lipid content after 1-16 days of storage for samples for different treatments: antioxidant free (grey), rosemary extract 0,5% (red long stippled) rosemary extract 0,05% (red short stippled), tocopherols 0,5% (blue long stippled) and tocopherols 0,05% (blue short stippled).

3.4 Lipid oxidation

3.4.1 Peroxide value

A diagram showing measured PV values for vacuum packed salmon at 4°C at day 1 and day 16 from batch 1 is shown in figure 3.5. The change in PV between 1 day to day 16 was significant (P<0,05) showing a clear process of lipid oxidation taking place. The values for day had a higher SDOM which may be caused by the method's low sensitivity, at only 0,5 meq peroxide/kg lipids.

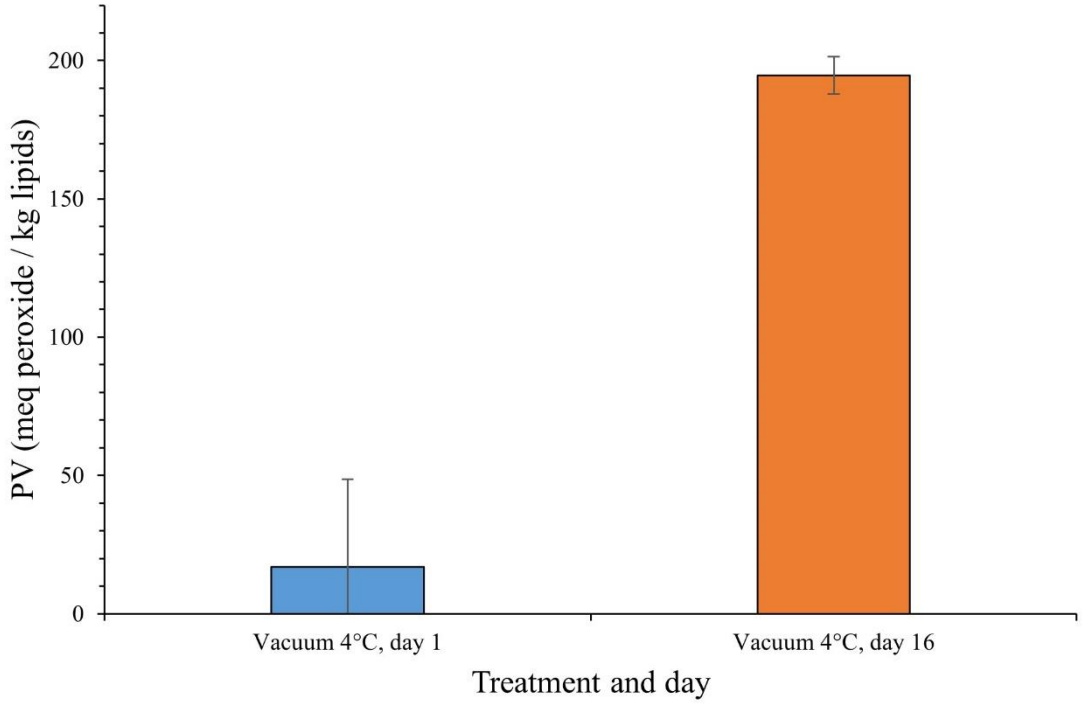

Figure 3.7: Peroxide values (meq peroxide/kg lipids) for vacuum packed salmon after 1 (blue) and 16 (red) day(s) of cold storage at 4°C. The error bars represent SDOM.

This does not give the full picture, however. As PV only shows primary oxidation products, which do to not contribute to rancidity (7) (46), this alone does not determine at which level of rancidity the produce is at. Since the change in TBARS between day 1 and day 16 in batch 1 is insignificant, as seen in section 3.4.1, days previous to day 16 should not have a higher PV as rise in TBARS values should cause a decrease in PV.

Figure 3.8 shows the PV for antioxidant free samples from day 1 and all treatments from day 16 from batch 2. Again, as seen in section, 3.4.2 the TBARS remains stable for these two days so PV is the main measurement of oxidation during this storage experiment.

There is a slight decrease in PV for samples treated with rosemary, regardless of concentration used. However, any difference between antioxidant free samples from day 16 and antioxidant treated samples was insignificant. This difference may be more pronounced given more samples or the study is given a longer time frame, and thereby given the rise of TBARS within the samples. However, this study cannot conclude anything regarding antioxidant effect on lipid oxidation. It should be noted that the significant is affect by the lack of samples. The error bars are based upon two measurements for the PV are based upon two measurements. Given more parallels, and thereby a larger pool of data, there may be a more significant difference between the treatment methods. The difference between PV from day 1 and day 16 for any treatment group is significant ($P<0,05$). This was obviously expected as PV usually increases early in the sample storage.

The PV value between antioxidant free samples from the batch 2 does not show any significant difference between the PV value for vacuum-packed samples at 4°C meaning the oxidation between to the experiments remain comparable.

The values obtained here are around 200 meq / kg lipids which is quite high for fish fats and is well above the threshold for what can be considered fit for human consumption. However, PV is generally highly empirical so comparisons between studies can be difficult (47). Regardless this value is way above the standard set by The European Pharacopeia (Ph.Eur) who set the quality parameter of PV at $PV \leq 10$ meq/kg (46). It should be noted, however, that PV over 100 meq/kg have been observed in Atlantic salmon (48) (23).

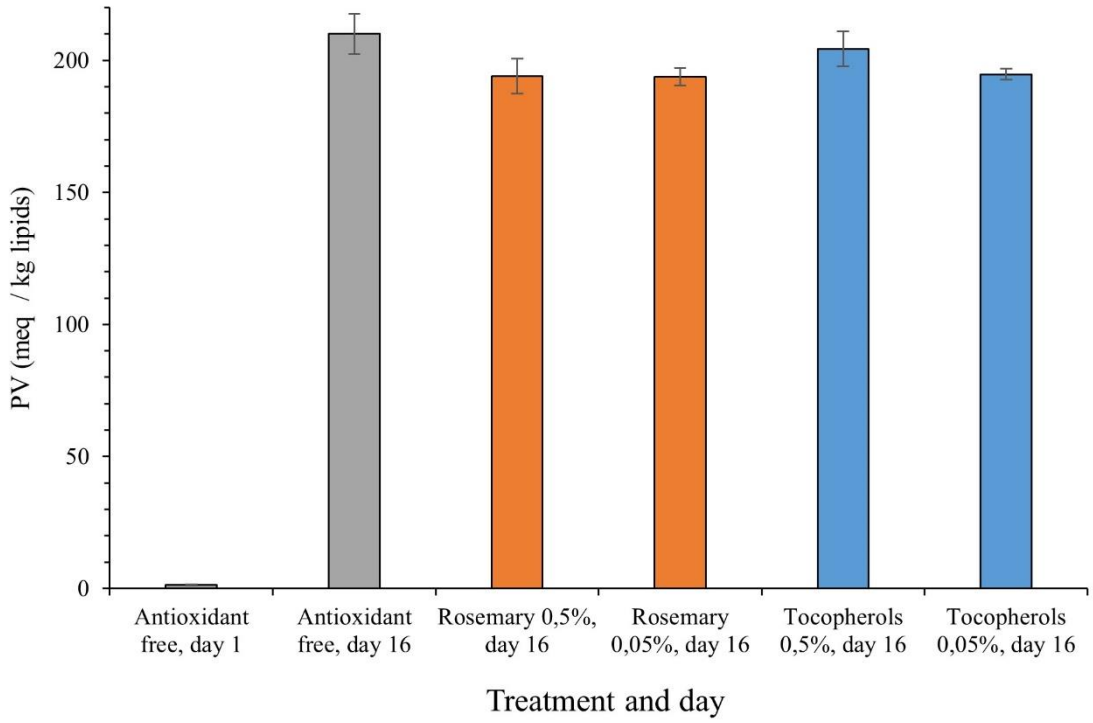

Figure 3.8: Peroxide values (meq peroxide/kg lipids) for antioxidant free samples (grey) from day 1 and 2, Rosemary 0,5% and 0,05% from day 16 (red), and tocopherols 0,5% and 0,05% from day 16 (blue). The error bars represent SDOM.

3.4.2 TBARS

Pervious studies of TBARS values in a similar time aspect as the ones conducted here did find a significant change in TBARS values between days, however not between vacuum and MAP. Pairing TBARS values with PV will give a greater picture of the overall quality of the samples between the start of the study to the end of the study. A diagram of showing the measured TBARS values is shown in figure 3.7 for vacuum-packed salmon at 4°C at day 1 and day 16. The difference in TBARS values between day 1 and day 16 was not significant.

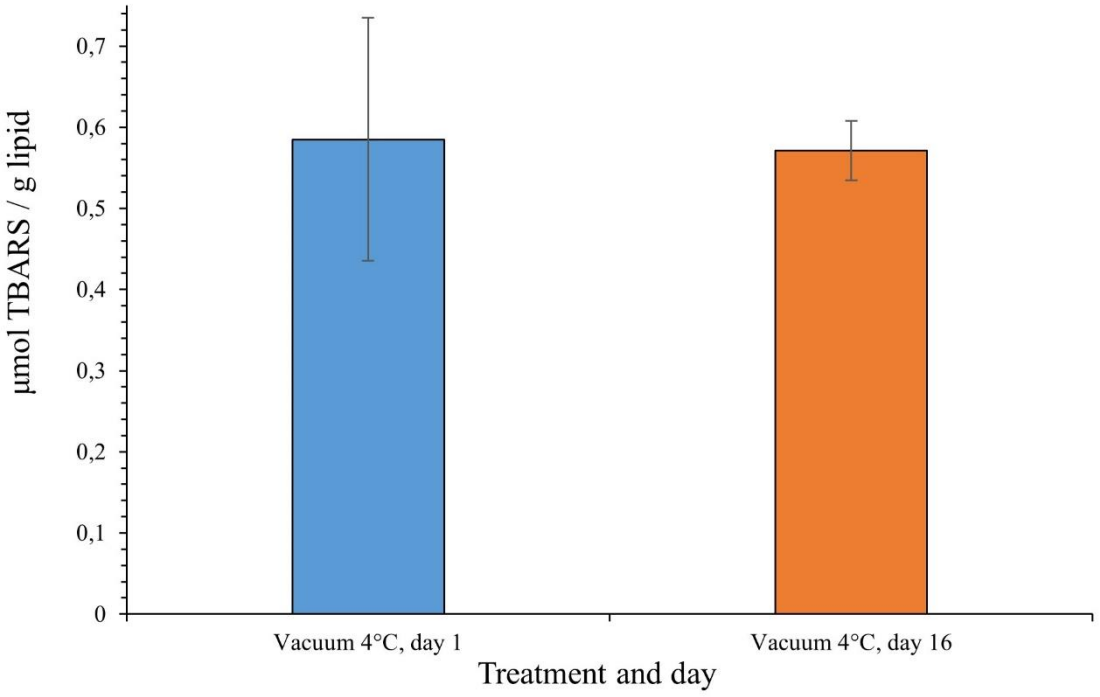

Figure 3.9: TBARS values (µmol TBARS/g lipid) for vacuum packed salmon after 1 (blue) and 16 (red) day(s) of cold storage at 4°C. The error bars represent SDOM.

This is contradictory to previous studies in which TBARS values have increased with time (37) (49). The PV values shows that the process of oxidation has well started, but the TBARS values show that there may not be significant change in rancidity. There should therefore not be any change in rancidity across any of the other storage methods as they should not be able to affect the concentration of TBARS further.

There could have been a change in TBARS for samples treated with antioxidants as they should terminate the autooxidation happening within the fat of the samples. Regardless, the amount of TBARS from day should be similar across all treatment methods. Therefore, there was only a need to test the antioxidant free samples from day 1 and test all treatment groups for day 16. The amount of TBARS per gram lipid for antioxidant free samples on day 1 and 16 as well as all treatment groups from day 16 is shown in figure 3.10.

Most samples treated with antioxidants show a decrease in amount of TBARS from day 16 compared to the day 1 sample, except for samples treated with 0,5% rosemary extract. This should not be possible as the amount of TBARS do not decrease during storage. However, any difference between antioxidants samples from day 16 and antioxidant free samples from day 1 was statistically insignificant.

The TBARS values obtained in this study are within values TBARS values from other studies dealing with Atlantic salmon (37) (50) (51) (52).There is, however, a difference significant difference in TBARS values between day 1 from batch 1 and 2 (P<0,05).

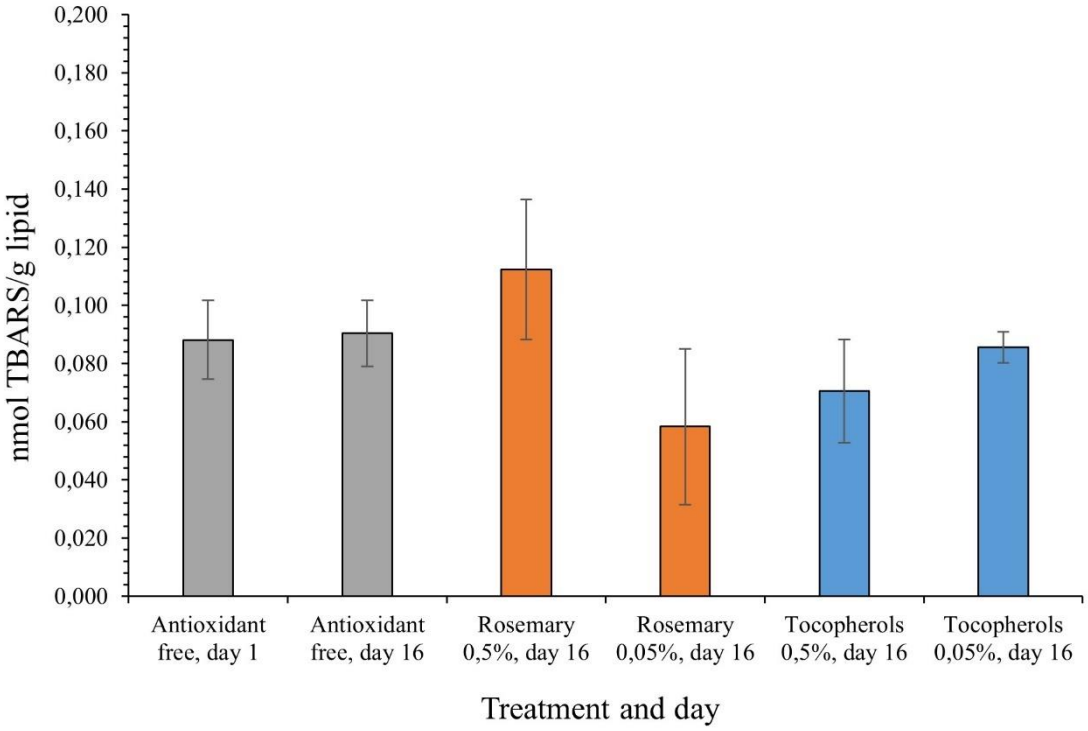

Figure 3.10: TBARS values (µmol TBARS/g lipid) for antioxidant free samples (grey) from day 1 and 2, Rosemary 0,5% and 0,05% from day 16 (red), and tocopherols 0,5% and 0,05% from day 16 (blue). The error bars represent SDOM.

3.5 Limits of the study and challenges

The study overall suffers from a lack of samples, where most SDOM are calculated from two parallel samples. Several measurements were done with only one test parallel causing SDOM to not be calculable.

3.5.1 Drip loss

Overall, the sampling method was good, however a more accurate analytical scale could have been used. As drip loss of fish meats may be dependent on pressure (53) changes in packaging pressure may uncertainties which may be controlled for. There is probably a reduction of CO_2 in the MAP packaged samples (38) causing less pressure, which may put them in favour vacuum packed samples which receive pressure from the packaging itself. These changes in pressure are on the other hand usually unavoidable.

3.5.2 Microbial Growth

For each plate number counted there was only one parallel as all samples only had one countable plate. Therefore, no SDOM is calculated for the samples. Several of the samples had a low plate count meaning they may be unreliable for accurate CFU/g. This happened due to the use of low concentration suspensions from stomacher homogenised samples. In particular MAP packed samples at 0°C suffered from this causing any distinctions between MAP and vacuum-packed samples at 0°C to be hard to verify. This did not affect the divergence between 4°C stored samples and 0°C samples. In batch 2 low plate counts were unavoidable as a majority of the plates counted were undiluted from the stomacher homogenised suspension.

Batch 2 suffered from what seems to be contamination, as previously mentioned in section 3.2. Contamination may also have affected the measurements for CFU in the natural salmon flora suspension. The suspension was plated out as undiluted, 10^{-1} dilution, 10^{-2} dilution and 10^{-3} dilution. All plates, except for 10^{-3} dilution, which had no observable colonies, was in practice uncountable as they had an overgrowth of bacteria. The plate count of 291 and 279 for 10^{-2} dilution may be highly inaccurate a there was probably more visible colonies that were left uncounted. The plate count of the 10^{-3} dilution plate was at 0 giving further evidence that the CFU/mL of the natural salmon flora suspension is highly inaccurate. This does not affect the results as at the very least the plate counts confirm there to be bacteria in the suspension carrying the red colonies characteristic from plates counted in batch 1 and later plates counted in batch 2.

3.5.3 Lipid oxidation

TBARS had to be repeated multiple times to get a satisfactory standard curve for nmol TEP against absorbance at 538 nm. This was due to the absorbance being way too high to fit in linear regression. Diluting the standard samples was also tried, however the samples had to be diluted 1:500 to reach absorbance readings within expected values. This happened despite trying several TEP solution made by different people all to be at 0,1 mM concentration. This was solved using a TEP solution made in 2018, which was the standard solution used for both standard curves shown in appendix A.4.2. Leakage was a problem while oiling the test-tubes causing unknown and unwanted concentrations of TEP and sample solutions. All test tubes were checked for leakage after boiling, and samples with an obvious loss of liquid was either discarded if the loss were great enough or noted down so any large differences between the leaked sample and its parallel could be explain.

As mentioned in section 3.4 the PV measured in this study is higher than what was expected. The reason for this remains unclear. Several of the PV values for the test parallels were similar, and the values from batch 1 and batch 2 are comparable and remains within similar PV. The result are however usable and clearly indicates an increase in PV the different days can still show differences between treatment methods. As this study does not really focus on the specific values lipid oxidation have to stay within for consumption, but rather the prolonging of shelf life and quality of salmon.

TBARS values, as mentioned in section 3.4, remains within expected values. The measured TBARS values are significantly different between batch 1 and batch 2, however TBARS should still focus on the change in values rather than the absolute values due to TBARS already existing in food without the compounds being secondary oxidation products (7).

3.6 Evaluation of antioxidant application

As there are few studies applying tocopherols directly to fish meats and few studies applying rosemary extracts to fish without the use of water, there were uncertainties in whether the method would work at all in this study. Ultimately the use of ethanol could kill any surface bacteria in the fish filets meaning that microbial analyses could be meaningless as the samples would be sterile. The use of a natural salmon flora suspension was used only to ensure the presence of bacteria in order to test the microbial growth. The control was treated with antioxidant free ethanol to ensure that when measuring the parameters in this study it would

be assumed that any changes was only due to the use of antioxidants and not the use of ethanol.

As the differences between control and antioxidant samples from batch 2 are small the application method worked in not discriminating any samples and that the treatment parallels in batch 2 remain comparable to each other. The colonies counted also retained the characteristic red colour in batch 2 found in batch 1. However, if the bacteria present in batch 2 is the same as the bacteria in batch 1 was not tested.

The only factor which could largely affect the usage of this application method in the future is the amount of antioxidants in the tissue after application. However, earlier studies have found rosemary to have an effect on lipid oxidation when applied to the fish in this manner and at similar concentrations for rainbow trout (19) and sardines (54). Since the final antioxidant content for the samples were not measured it is uncertain whether the lack of significant result was due to the lack of antioxidants dissolving in the fish tissue.

4 Conclusion

Drip loss increased during storage for vacuum-packed samples where MAP samples from batch 1 had no significant increase in drip loss during storage. All samples from batch 2 had an increasing drip loss with storage time. No differentiation between antioxidant treated samples and the control was observed.

Samples stored at 0°C increased their shelf life significantly compared to samples stored at 4°C. All samples packed in MAP at 0°C remained within consumption limit throughout the storage experiment, all samples from batch 2 being treated with ethanol the CFU/g for the samples may be influenced by said treatment. There was no differentiation between samples treated with different antioxidants compared to each other nor the control. Fat content was similar between batch 1 and 2, though somewhat higher on average for batch 2, but less spread out in terms of extremes of low and high values.

In terms lipid oxidation no treatment had significant change in neither PV nor TBARS values compared with each other. PV changed significantly between day 1 and day 16. No significant change was measured in TBARS values neither between day nor by treatment method.

In sum the current study found differences in quality and shelf life of Atlantic salmon dependent on whether the sample was MAP or vacuum-packed, and temperature. No change in shelf life nor quality was dependent on the addition of antioxidants.

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Appendix Experimental data and sample calculations

A.1 Drip Loss

A.1.1 Drip loss example calculation

Table A.1 shows raw data and results from day 16 of the first storage experiment. Table A.2 shows raw data from day 16 of the second storage experiment. Equation A.1 shows a sample calculation of drip loss.

$$Drip\ loss\ (\%) = \frac{(54{,}71\ g - 47{,}02\ g) - 5{,}28\ g}{47{,}02 g} * 100\% = 5{,}13\% \quad \text{(A.1)}$$

Table A.1: Raw data from day 16 of the first storage experiment, including weight of packed samples (g), weight of fish samples (g), weight of dried packaging (g) and the final drip loss (%) for each storage method and corresponding parallel.

Treatment method, parallel	Weight of packed sample (g)	Weight of fish sample (g)	Weight of dried packaging (g)	Drip loss (%)
Vacuum 4°C, 1	54,71	47,02	5,28	5,13
Vacuum 4°C, 2	54,23	47,20	5,29	3,69
Vacuum 0°C, 1	56,76	49,91	5,25	3,21
Vacuum 0°C, 2	52,52	44,33	5,29	6,54
MAP 4°C, 1	61,54	52,06	8,97	0,98
MAP 4°C, 2	59,23	49,24	9,27	1,46
MAP 0°C, 1	58,24	48,58	9,20	0,95
MAP 0°C, 2	59,27	49,40	9,26	1,23

Table A.2: Raw data from day 16 of the second storage experiment, including weight of packed samples (g), weight of fish samples (g), weight of dried packaging (g) and the final drip loss (%) for each treatment method and corresponding parallel.

Treatment method, parallel	Weight of packed sample (g)	Weight of fish sample (g)	Weight of dried packaging (g)	Drip loss (%)
Antioxidant free, 1	53,19	43,34	8,88	2,24
Antioxidant fee, 2	54,93	45,33	8,85	1,65
Rosemary 0,5%, 1	54,12	44,29	9,48	0,79
Rosemary 0,5%, 2	55,70	44,61	9,65	3,23
Rosemary 0,05%, 1	51,42	41,54	8,84	2,50
Rosemary 0,05%, 2	51,21	41,49	8,86	2,07
Tocopherols 0,5%, 1	57,20	47,39	8,91	1,90
Tocopherols 0,5%, 2	54,43	44,21	8,82	3,17
Tocopherols 0,05%, 1	50,32	41,07	8,84	1,00
Tocopherols 0,05%, 2	56,67	47,01	8,84	1,74

A.1.2 Drip loss raw data

Table A.3 shows the drip loss for every measured day for each storage method and parallel. Table A.4 shows the drip loss for every treatment method for every measured day and parallel.

Table A.3: Drip loss for every storage method for the first storage experiment and their corresponding parallel, for day 1 (D.1), day 3 (D.3), day 6 (D.6), day 8 (D.8), day 10 (D.10), day 13 (D.13) and day 16 (D.16).

	Drip loss (%)						
	D.1	**D.3**	**D.6**	**D.8**	**D.10**	**D.13**	**D.16**
Vacuum 4°C, 1	0,73	0,66	3,06	2,82	2,15	2,94	5,13
Vacuum 4°C, 2	0,00	1,29	1,45	1,79	3,75	7,08	3,69
Vacuum 0°C, 1	0,95	2,34	1,82	2,53	2,55	3,01	3,21
Vacuum 0°C, 2	1,01	2,95	1,14	1,95	1,67	7,46	6,54
MAP 4°C, 1	0,90	0,51	2,28	0,89	1,14	1,36	0,98
MAP 4°C, 2	0,71	0,75	0,90	1,65	1,01	0,95	1,46
MAP 0°C, 1	1,08	0,52	0,78	0,79	0,65	1,00	0,95
MAP 0°C, 2	0,54	0,50	2,62	1,27	0,81	0,53	1,23

Table A.4: Drip loss for every treatment method for the second storage experiment and their corresponding parallel, for day 1 (D.1), day 3 (D.3), day 6 (D.6), day 8 (D.8), day 10 (D.10), day 13 (D.13) and day 16 (D.16).

	Drip loss (%)						
	D.1	**D.3**	**D.6**	**D.8**	**D.10**	**D.13**	**D.16**
Antioxidant free, 1	0,39	0,42	1,92	0,89	1,12	1,24	2,24
Antioxidant free, 2	0,27	0,80	0,97	1,07	3,54	3,04	1,65
Rose. 0,5%, 1	1,10	0,53	1,62	0,59	1,52	0,94	0,79
Rose- 0,5%, 2	0,26	0,98	1,00	0,92	4,37	1,24	3,23
Rose. 0,05%, 1	1,13	1,07	0,89	1,69	1,02	1,87	2,50
Rose. 0,05%, 2	0,15	1,73	1,11	1,20	2,06	2,73	2,07
Toco. 0,5%, 1	0,33	2,15	1,02	1,28	1,17	2,46	1,90
Toco. 0,5%, 2	0,50	0,65	0,38	1,45	1,09	0,98	3,17
Toco. 0,05%, 1	0,28	0,78	0,62	2,24	2,17	1,86	1,00
Toco. 0,05%, 2	0,51	0,67	1,11	1,00	2,07	5,22	1,74

A.2 Microbial growth

Table A.3 and A.4 shows raw data for microbial growth, including plate number, number of dilutions and sample weight for each treatment method from the first and the second storage experiment, respectively.

Table A.5: Microbial growth for the first storage experiment, including sample weight (g), numbers of dilutions, numbers of counted colonies for every treatment and parallel.

	Treatment, parallel	Sample weight (g)	Number of 1:10 dilutions	Number of colonies
Day 1	Vacuum 4°C, 1	10,13	1	1
	Vacuum 4°C, 2	10,63	1	11
	Vacuum 0°C, 1	9,10	1	2
	Vacuum 0°C, 2	10,01	1	2
	MAP 4°C, 1	9,37	1	2
	MAP 4°C, 2	9,94	1	38
	MAP 0°C, 1	9,07	1	1
	MAP 0°C, 2	9,26	1	5
Day 3	Vacuum 4°C, 1	10,50	1	17
	Vacuum 4°C, 2	10,24	1	37
	Vacuum 0°C, 1	10,14	1	5
	Vacuum 0°C, 2	10,63	1	9
	MAP 4°C, 1	10,85	1	15
	MAP 4°C, 2	10,24	1	8
	MAP 0°C, 1	10,06	1	0
	MAP 0°C, 2	10,76	1	1
Day 6	Vacuum 4°C, 1	10,17	4	17
	Vacuum 4°C, 2	10,58	2	44
	Vacuum 0°C, 1	10,80	1	78
	Vacuum 0°C, 2	9,83	1	12
	MAP 4°C, 1	10,30	2	13
	MAP 4°C, 2	10,20	2	24
	MAP 0°C, 1	10,49	1	9
	MAP 0°C, 2	10,41	1	3
Day 8	Vacuum 4°C, 1	10,20	3	115
	Vacuum 4°C, 2	9,42	3	65
	Vacuum 0°C, 1	9,57	1	61
	Vacuum 0°C, 2	10,13	1	97
	MAP 4°C, 1	9,30	2	95
	MAP 4°C, 2	10,65	3	53
	MAP 0°C, 1	9,36	1	107
	MAP 0°C, 2	9,79	1	13

Day 10	Vacuum 4°C, 1	11,18	5	67
	Vacuum 4°C, 2	10,69	5	35
	Vacuum 0°C, 1	9,48	2	17
	Vacuum 0°C, 2	9,30	2	7
	MAP 4°C, 1	9,74	5	33
	MAP 4°C, 2	10,09	4	121
	MAP 0°C, 1	10,38	2	2
	MAP 0°C, 2	9,33	2	6
Day 13	Vacuum 4°C, 1	9,34	5	76
	Vacuum 4°C, 2	9,09	5	43
	Vacuum 0°C, 1	9,55	3	4
	Vacuum 0°C, 2	9,05	4	8
	MAP 4°C, 1	9,11	6	21
	MAP 4°C, 2	9,45	5	83
	MAP 0°C, 1	9,19	3	1
	MAP 0°C, 2	9,08	3	2
Day 16	Vacuum 4°C, 1	9,56	6	18
	Vacuum 4°C, 2	10,00	6	28
	Vacuum 0°C, 1	9,38	3	15
	Vacuum 0°C, 2	9,81	4	25
	MAP 4°C, 1	9,67	6	28
	MAP 4°C, 2	9,40	5	110
	MAP 0°C, 1	9,10	3	4
	MAP 0°C, 2	9,55	3	6

Table A.6: Microbial growth for the first storage experiment, including sample weight (g), numbers of dilutions, numbers of counted colonies for every treatment and parallel.

	Treatment, parallel	Sample weight (g)	Number of 1:10 dilutions	Number of colonies
Day 1	Antioxidant free, 1	9,91	0	6
	Antioxidant fee, 2	10,43	1	117
	Rosemary 0,5%, 1	9,50	0	15
	Rosemary 0,5%, 2	10,20	1	46
	Rosemary 0,05%, 1	10,66	1	29
	Rosemary 0,05%, 2	9,90	0	129
	Tocopherols 0,5%, 1	10,27	0	62
	Tocopherols 0,5%, 2	10,29	0	102
	Tocopherols 0,05%, 1	10,47	1	32
	Tocopherols 0,05%, 2	9,64	3	77
Day 3	Antioxidant free, 1	9,2	0	8
	Antioxidant fee, 2	9,36	0	2
	Rosemary 0,5%, 1	9,21	0	32
	Rosemary 0,5%, 2	9,64	0	8
	Rosemary 0,05%, 1	9,40	0	7
	Rosemary 0,05%, 2	9,80	0	10
	Tocopherols 0,5%, 1	9,57	0	0
	Tocopherols 0,5%, 2	9,47	0	1
	Tocopherols 0,05%, 1	9,88	0	15
	Tocopherols 0,05%, 2	9,38	0	3
Day 6	Antioxidant free, 1	9,46	0	68
	Antioxidant fee, 2	10,49	0	13
	Rosemary 0,5%, 1	9,08	0	14
	Rosemary 0,5%, 2	9,36	0	8
	Rosemary 0,05%, 1	9,63	1	32
	Rosemary 0,05%, 2	10,01	0	4
	Tocopherols 0,5%, 1	9,14	0	2
	Tocopherols 0,5%, 2	9,24	0	9
	Tocopherols 0,05%, 1	9,50	0	5
	Tocopherols 0,05%, 2	9,58	0	8
Day 8	Antioxidant free, 1	10,23	0	18
	Antioxidant fee, 2	10,36	0	44
	Rosemary 0,5%, 1	9,62	0	6
	Rosemary 0,5%, 2	9,68	0	11
	Rosemary 0,05%, 1	10,13	0	48
	Rosemary 0,05%, 2	9,73	0	5
	Tocopherols 0,5%, 1	9,05	0	10
	Tocopherols 0,5%, 2	9,94	0	5
	Tocopherols 0,05%, 1	9,08	0	19
	Tocopherols 0,05%, 2	10,29	0	4

Day 10	Antioxidant free, 1	9,13	0	14
	Antioxidant fee, 2	9,26	0	23
	Rosemary 0,5%, 1	9,73	0	41
	Rosemary 0,5%, 2	9,47	0	16
	Rosemary 0,05%, 1	9,80	0	24
	Rosemary 0,05%, 2	9,08	0	8
	Tocopherols 0,5%, 1	10,00	0	4
	Tocopherols 0,5%, 2	9,39	0	19
	Tocopherols 0,05%, 1	10,09	0	22
	Tocopherols 0,05%, 2	9,92	0	28
Day 13	Antioxidant free, 1	9,24	0	24
	Antioxidant fee, 2	9,79	0	81
	Rosemary 0,5%, 1	10,29	0	120
	Rosemary 0,5%, 2	10,44	0	89
	Rosemary 0,05%, 1	10,84	0	15
	Rosemary 0,05%, 2	9,58	0	92
	Tocopherols 0,5%, 1	10,83	0	62
	Tocopherols 0,5%, 2	9,82	0	43
	Tocopherols 0,05%, 1	9,43	0	79
	Tocopherols 0,05%, 2	9,62	0	110
Day 16	Antioxidant free, 1	10,53	0	81
	Antioxidant fee, 2	9,43	1	41
	Rosemary 0,5%, 1	9,78	0	8
	Rosemary 0,5%, 2	9,17	0	176
	Rosemary 0,05%, 1	10,40	0	84
	Rosemary 0,05%, 2	9,45	0	76
	Tocopherols 0,5%, 1	9,77	0	115
	Tocopherols 0,5%, 2	10,33	0	85
	Tocopherols 0,05%, 1	9,64	0	172
	Tocopherols 0,05%, 2	9,74	0	79

A.3 Total lipid content

A.3.1 Lipid content example calculation

Table A.7 shows raw data for lipid content measurement for day 16 of the first experiment. Table A.8 shows raw data for lipid content measurement for day 16 of the second experiment. Equation A.2 shows a sample calculation of lipids per mL chloroform extract. Equation A.3 shows sample calculation of lipid content per gram sample.

$$Lipids\ per\ mL\ extract = \frac{11{,}2511g - 11{,}1773\ g}{2\ mL} = 0{,}0369\ g/mL \quad \text{(A.2)}$$

$$Lipid\ content\ (\%) = \frac{0{,}0369\ g/mL * 40\ mL}{10{,}36\ g} * 100\% = \ 14{,}25\% \quad \text{(A.3)}$$

Table A.7: raw data for measurement of lipid content from day 16 of the first experiment, including sample weight (g), weight of test tubes without lipids (g), weight of test tubes with lipids (g) and final lipid per mL extract (g/ml) for each treatment method and parallel.

Treatment method, parallel	Sample weight (g)	Weight of test tube w/o lipids (g)	Weight of test tubes w/ lipids (g)	Lipids/mL extract (g/mL)
Vacuum 4°C, 1	10,36	11,1773	11,2511	0,0369
Vacuum 4°C, 2	10,36	10,9578	10,9865	0,0143
Vacuum 0°C, 1	10,40	12,2855	12,3240	0,0192
Vacuum 0°C, 2	10,37	10,7262	10,8102	0,0420
MAP 4°C, 1	9,82	10,8875	10,9995	0,0560
MAP 4°C, 2	9,76	11,1010	11,1875	0,0432
MAP 0°C, 1	9,77	11,0854	11,1911	0,0529
MAP 0°C, 2	9,76	11,1710	11,2121	0,0206

Table A.8: raw data for measurement of lipid content from day 16 of the first experiment, including sample weight (g), weight of test tubes without lipids (g), weight of test tubes with lipids (g) and final lipid per mL extract (g/ml) for each treatment method and parallel.

Treatment method, parallel	Sample weight (g)	Weight of test tube w/o lipids (g)	Weight of test tubes w/ lipids (g)	Lipids/mL extract (g/mL)
Antioxidant free, 1	9,35	10,8455	10,9373	0,0459
Antioxidant free, 2	10,29	10,8762	10,9809	0,0523
Rose. 0,5%, 1	10,93	11,1736	11,2606	0,0435
Rose. 0,5%, 2	9,85	11,1564	11,2924	0,0680
Rose. 0,05%, 1	10,07	10,9427	11,0692	0,0633
Rose. 0,05%, 2	10,53	10,8344	10,8914	0,0285
Toco. 0,5%, 1	9,86	10,9064	11,0241	0,0589
Toco. 0,5%, 2	10,21	10,8317	10,9069	0,0376
Toco. 0,05%, 1	9,93	11,2973	11,3943	0,0485
Toco. 0,05%, 2	10,54	11,1706	11,2717	0,0505

A.3.2 Lipids per mL chloroform extract raw data

Table A.9 shows lipid per gram chloroform extract for every parallel for every day from the first experiment. Table A. 10 shows lipid per gram chloroform extract for every parallel for every day from the second experiment.

Table A.9: Lipid per gram chloroform extract (g/mL) for every storage method for the first storage experiment and their corresponding parallel, for day 1 (D.1), day 3 (D.3), day 6 (D.6), day 8 (D.8), day 10 (D.10), day 13 (D.13) and day 16 (D.16).

	Lipid per gram chloroform extract (g/mL)						
	D.1	**D.3**	**D.6**	**D.8**	**D.10**	**D.13**	**D.16**
Vacuum 4°C, 1	0,0242	0,0627	0,0248	0,0386	0,0197	0,0113	0,0369
Vacuum 4°C, 2	0,0132	0,0174	0,0597	0,0172	0,0229	0,0357	0,0143
Vacuum 0°C, 1	0,0301	0,0526	0,0400	0,0536	0,0297	0,0303	0,0192
Vacuum 0°C, 2	0,0354	0,0141	0,0600	0,0074	0,0453	0,0426	0,0420
MAP 4°C, 1	0,0217	0,0134	0,0469	0,0210	0,0304	0,0261	0,0560
MAP 4°C, 2	0,0126	0,0193	0,0349	0,0081	0,0493	0,0469	0,0432
MAP 0°C, 1	0,0199	0,0253	0,0205	0,0276	0,0390	0,0560	0,0529
MAP 0°C, 2	0,0347	0,0274	0,0515	0,0188	0,0260	0,0286	0,0206

Table A.10: Lipid per gram chloroform extract (g/mL) for every storage method for the second storage experiment and their corresponding parallel, for day 1 (D.1), day 3 (D.3), day 6 (D.6), day 8 (D.8), day 10 (D.10), day 13 (D.13) and day 16 (D.16).

	Lipid per gram chloroform extract (g/mL)						
	D.1	**D.3**	**D.6**	**D.8**	**D.10**	**D.13**	**D.16**
Antioxidant free, 1	0,0341	0,0570	0,0365	0,0464	0,0576	0,0422	0,0459
Antioxidant free, 2	0,0387	0,0339	0,0277	0,0386	0,0264	0,0524	0,0523
Rose. 0,5%, 1	0,0284	0,0277	0,0419	0,0251	0,0469	0,0495	0,0435
Rose. 0,5%, 2	0,0245	0,0459	0,0264	0,0528	0,0541	0,0538	0,0680
Rose. 0,05%, 1	0,0433	0,0375	0,0104	0,0217	0,0583	0,0373	0,0633
Rose. 0,05%, 2	0,0379	0,0315	0,0219	0,0460	0,0301	0,0510	0,0285
Toco. 0,5%, 1	0,0480	0,0471	0,0328	0,0330	0,0382	0,0346	0,0589
Toco. 0,5%, 2	0,0215	0,0325	0,0213	0,0380	0,0422	0,0591	0,0376
Toco. 0,05%, 1	0,0274	0,0175	0,0382	0,0371	0,0602	0,0302	0,0485
Toco. 0,05%, 2	0,0457	0,0368	0,0347	0,0353	0,0236	0,0616	0,0505

A.4 Lipid oxidation

A.4.1 PV

Table A.11 shows the raw data for PV measurements for the first storage experiment. Table A.12 shows the raw data for PV measurements for the second storage experiment. Equation A.4 shows a sample calculation of PV from raw data.

$$PV = \frac{0{,}1610\ mL - 0{,}0221\ mL * 0{,}01\ M * 1000}{1{,}150\ g} = 1{,}208\ meq\ /\ kg \quad \text{(A.4)}$$

Table A.11: Raw data from PV measurements for vacuum treated samples at 4°C, including volume of Na_2SO_3 for the sample as well as for the corresponding blank sample. Each measurement was done in two parallels, both shown in the table as test parallel 1 and test parallel 2.

	Test parallel 1		Test parallel 2	
Treatment, parallel and day	Volume Na2SO3 Sample	Volume Na2SO3 Blank	Volume Na2SO3 Sample	Volume Na2SO3 Blank
Vacuum 4°C I, day 1	0,1610	0,0221	0,1574	0,0221
Vacuum 4°C II, day 1	0,0823	0,0221	7,5777	0,0221
Vacuum 4°C I, day 16	19,7586	0,0221	21,0827	0,0221
Vacuum 4°C II, day 16	19,7282	0,0221	21,6460	0,0221

Table A.12: Raw data from PV measurements for antioxidant treated samples at day 16 and antioxidant free samples from day 1 and 16. The table includes volume of Na_2SO_3 for the sample as well as for the corresponding blank sample. Each measurement was done in two parallels, both shown in the table as test parallel 1 and test parallel 2.

Treatment and day	Parallel 1		Parallel 2	
	Volume Na2SO3 Sample	Volume Na2SO3 Blank	Volume Na2SO3 Sample	Volume Na2SO3 Blank
Antioxidant free, day 1	0,1397	0,0288	0,1881	0,0233
Antioxidant free, day 16	21,1335	0,0288	21,3119	0,0288
Rose. 0,5%, day 16	19,9133	0,0288	19,7074	0,0288
Rose. 0,05%, day 16	21,0726	0,0288	21,2158	0,0233
Toco. 0,5%, day 16	21,1337	0,0288	20,3921	0,0288
Toco. 0,05%, day 16	19,9266	0,0288	21,4145	0,0233

A.4.2 TBARS

Figure A.1 and A.2 shows the standard curve for nmol TEP over absorbance at 538 nm for the first and second storage experiment, respectively. Table A.13 shows the raw data for the TBARS values for the first storage experiment. Table A.14 shows the raw data for TBARS values for the second storage experiment. Equation A.5 shows an example calculation of TBARS value from raw data.

$$TBARS\ value = \frac{0{,}062 - 0{,}00511}{0{,}0134 * 0{,}00968\ g * 1000} = 0{,}438\ nmol\ TBARS/\ g\ lipid \quad \text{(A.5)}$$

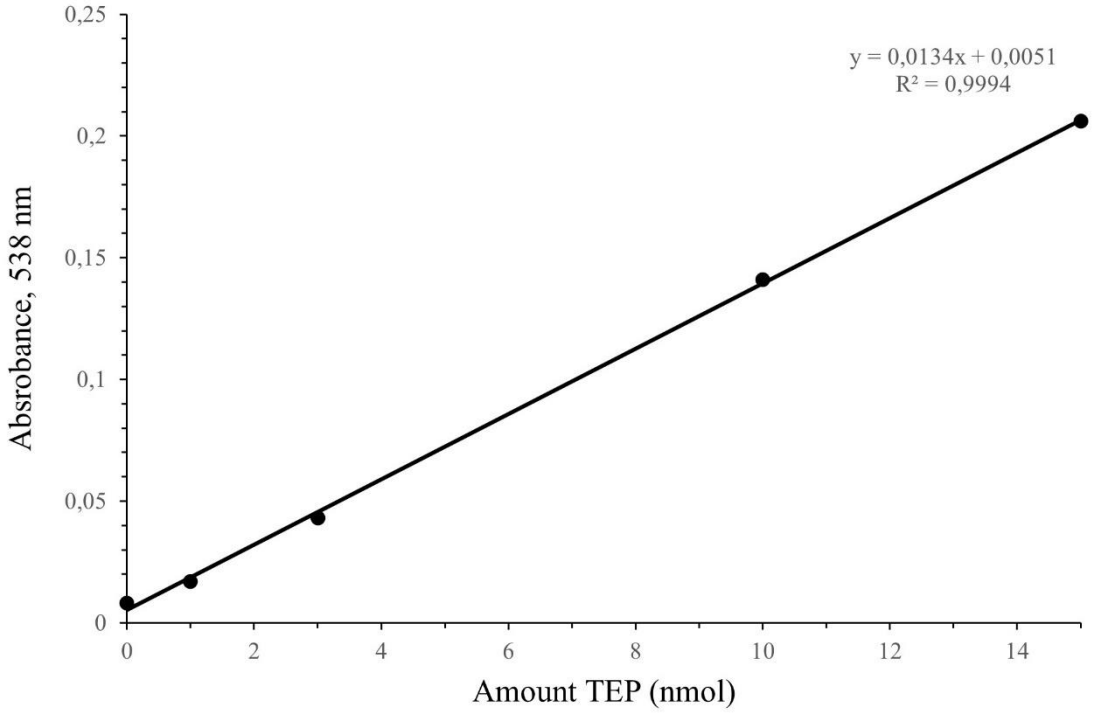


Figure A.1: Standard curve for nmol TEP against absorbance at 538 nm, for the first storage experiment.

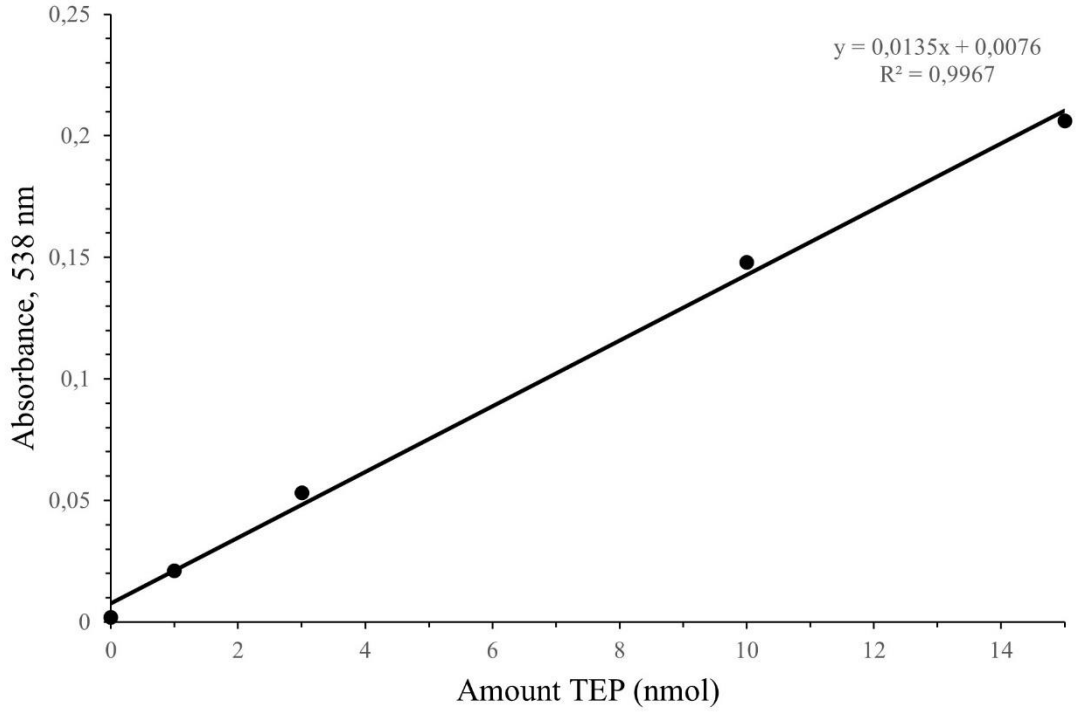


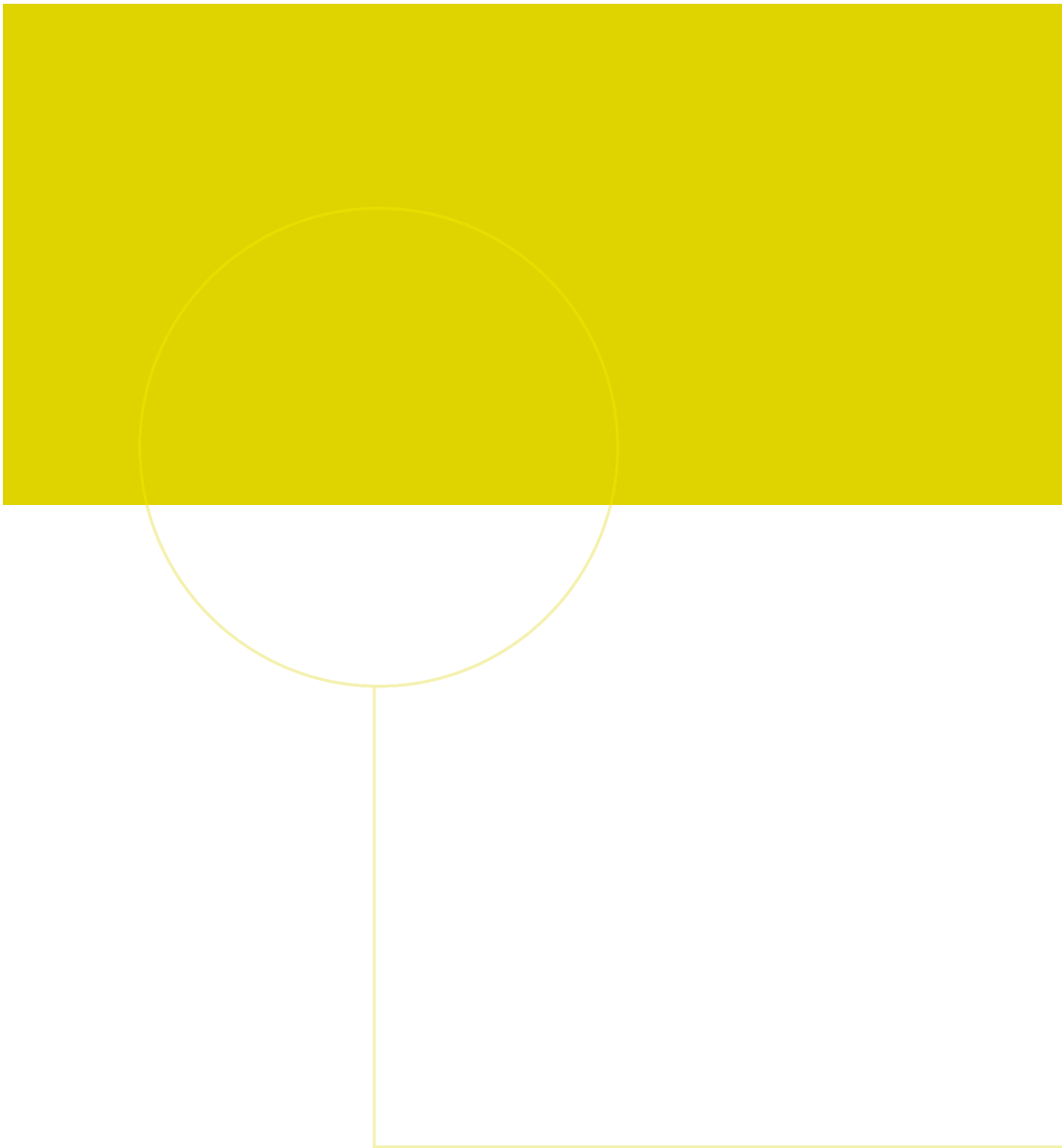
Figure A.2: Standard curve for nmol TEP against absorbance at 538 nm, for the second storage experiment.

Table A.13: Raw data from TBARS value measurements for vacuum treated samples at 4°C, with corresponding absorbances at 538. Each measurement was done in two parallels, both shown in the table as test parallel 1 and test parallel 2.

	Test parallel 1	**Test parallel 2**
Treatment, parallel, day	**Absorbance, 538 nm**	**Absorbance, 538 nm**
Vacuum 4°C I, day 1	0,062	0,067
Vacuum 4°C II, day 1	0,054	0,057
Vacuum 4°C, I, day 16	0,124	0,121
Vacuum 4°C, II, day 16	0,045	0,050

Table A.14: Raw data from TBARS value measurements for antioxidant treated samples at day 16 and antioxidant free samples from day 1 and 16 with corresponding absorbances at 538 nm. Each measurement was done in two parallels, both shown in the table as test parallel 1 and test parallel 2.

Treatment, day	Test parallel 1 Absorbance, 538 nm	Test parallel 2 Absorbance, 538 nm
Antioxidant free, day 16	0,028	0,032
Rose. 0,5%, day 16	0,038	0,030
Rose. 0,05%, day 16	0,034	0,021
Toco. 0,5%, day 16	0,034	0,026
Toco. 0,05%, day 16	0,029	0,031
Antioxidant free, day 1	0,024	0,028



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