

Andrea Hagen

The Potential of Biobased Materials Combined with Soluble Gas Stabilisation in Packaging of Atlantic Salmon (*Salmo salar* L.) Fillets

Master's thesis in Food and Technology

Supervisor: Jørgen Lerfall

Co-supervisor: Anita N. Jakobsen

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Abstract

In the wake of a growing concern towards sustainability issues globally and the carbon impact of petroleum-based food packaging materials, there has become an increasing focus upon utilizing materials that are both biobased and biodegradable. The challenges for these materials to become a conventional part of the industry have been related to cost, material processing and performance in terms of maintaining product quality in line with petroleum-based materials. Atlantic salmon is a perishable product with limited shelf-life, causing several requirements for maintaining quality during storage. Salmon has been essential to Norway's export economy for several decades, and will most likely be of great importance for Norway in the future.

This thesis aimed to compare two biobased and biodegradable plastic materials with different barrier properties (low and high barrier) to a conventional petroleum-based plastic material to see their effect on quality and shelf-life to fresh salmon fillets during 20 days of storage (4 °C). The goal was to maintain the quality and shelf-life of portioned Atlantic salmon fillets packaged in biobased and biodegradable materials using a petroleum-based material as control. A biobased single-layer film made of cassava root and corn derivatives blended with polybutylene adipate-*co*-terephthalate (BioLB) and a high barrier duplex film made of cellulose laminated to biobased polybutylene succinate (BioHB) were used, along with a petroleum-based polyamide/polyethylene film (PA/PE). This study investigated two packaging methods; vacuum packaging (VP) and modified atmosphere (MA) packaging (60 % CO₂, 40 % N₂). Soluble gas stabilisation (SGS, 18 hrs in 100 % CO₂), as a pre-treatment, was used as an experimental factor within each packaging method to see if it had any further advantages for the fish's quality. Analyses in this study were a microbiological parameter (total viable count), four physiochemical parameters (pH, drip loss, colour and texture), a biochemical parameter (degradation of adenosine triphosphate), and measurements of headspace gas during MA packaging.

Based on all analyses conducted in this study, it can be concluded that BioLB is not a suitable material for MA packaging due to its poor barrier properties. However, the results from VP can indicate that BioLB is somewhat suitable, even though the control group of PA/PE performed better in general. Contrarily, the high barrier properties of BioHB gave acceptable results for maintaining quality for Atlantic salmon in both MA packaging and VP, and is therefore suitable as a biobased and biodegradable packaging material for Atlantic salmon fillets.

Sammendrag

Følgelig av en globalt økende bekymring for problemstillinger rundt bærekraft, og karbonpåvirkningen av petroleumsbaserte materialer, har det blitt et utvidet fokus på å produsere og utnytte materialer som både er biobaserte og biologisk nedbrytbare. De utfordrende faktorene for at disse materialene kan bli en konvensjonell del av industrien har i all hovedsak vært kostnader, materialprosessering og ytelsen i form av å opprettholde produktkvalitet og holdbarhet på lik linje som petroleumsbaserte materialer. Atlantisk laks er et lett bedervelig produkt med begrenset holdbarhet, som leder til en rekke krav for opprettholdelse av kvalitet gjennom lagring. Laks har vært viktig for Norges eksportøkonomi i flere tiår, og vil mest antakelig fortsette å være viktig for Norge i årene fremover.

Hensikten med denne oppgaven var å sammenligne to biobaserte og biologisk nedbrytbare plastikkmaterialer med forskjellige barriereegenskaper (lav og høy barriere) opp mot et konvensjonelt petroleumsbasert plastikkmateriale for å se deres effekt på kvalitet og holdbarhet til ferske laksefileter under lagring (4 °C) på 20 dager. Målet var å opprettholde kvaliteten og holdbarheten til porsjonerte atlantiske laksefileter pakket i biobaserte og biologisk nedbrytbare materialer ved bruk av et petroleumsbasert materiale som kontroll. En biobasert enkeltlagsfilm laget av kassavarot og maisderivater blandet med polybutylenadipat-*co*-tereftalat (BioLB) og en høybarriere dupleksfilm laget av cellulose som er laminert til biobasert polybutylensuccinat (BioHB) ble brukt, sammen med en petroleumsbasert polyamid/polyetylen film (PA/PE). Studien undersøkte to emballasjemetoder; vakuumpakking (VP) og modifisert atmosfære (MA) pakking (60 % CO₂, 40 % N₂). «Soluble gas stabilisation» (SGS, 18t med 100 % CO₂), som forbehandling, ble brukt som en eksperimentell faktor i hvert av emballasjemetodene, for å se om det hadde ytterligere fordeler for fiskens kvalitet. Analysene som ble utført var én mikrobiologisk parameter (total kim), fire fysiokjemiske parametere (pH, drypptap, farge og tekstur), én biokjemisk parameter (nedbrytning av adenosintrifosfat) og målinger av gassblanding under lagring i MA.

Basert på analysene som ble utført i denne studien, kan det konkluderes med at BioLB ikke var et egnet materiale for lagring i MA, på grunn av dens dårlige barriereegenskaper. Resultatene fra VP kan imidlertid indikere at BioLB var noe passende, selv om kontrollgruppen for PA/PE presterte bedre. I motsetning til dette, ga de høye barriereegenskapene i BioHB akseptable resultater for opprettholdelse av kvaliteten for laks i både MA pakking og VP, og er dermed et passende biobasert og biologisk nedbrytbart emballasjemateriale for atlantisk laks.

Preface and Acknowledgments

This thesis covers 45 ECTS points and marks the completion of the MSc programme for Food and Technology at the Department of Biotechnology and Food Science (IBM), Faculty of Natural Science (NV) at the Norwegian University of Science and Technology (NTNU).

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Abbreviations

ADP	Adenosine Diphosphate	N₂	Nitrogen
AMP	Adenosine Monophosphate	NPN	Non-Protein Nitrogenous
APC	Aerobic Plate Counts	O₂	Oxygen
ATP	Adenosine Triphosphate	PA	Polyamide
BF	Breaking Force		
BioHB	Biobased with High Barrier	PBS	PolyButylene Succinate
BioLB	Biobased with Low Barrier	PBAT	PolyButylene Adipate-co-Terephthalate
BioPBS	Biobased PolyButylene Succinate	PE	Polyethylene
CFU	Colony-forming Unit	PET	Polyethylene Terephthalate
CO₂	Carbon dioxide	PFMG	Professional Food Microbiology Group
CO₂PC	Carbon Dioxide Permeability Coefficient	OPC	Oxygen Permeability Coefficient
CO₂TR	Carbon Dioxide Transmission Rate	OTR	Oxygen Transmission Rate
CPET	Crystalline Polyethylene Terephthalate	RF	Resistance Force
EVOH	Ethylene Vinyl Alcohol	rPET	Recycled Polyethylene Terephthalate
GLM	General Linear Model	SGS	Soluble Gas Stabilisation
GMP	Good Manufacturing Practice	SSO	Specific Spoilage Organisms
HPLC	High-Performance Liquid Chromatography	TMA	Trimethylamine
Hx	Hypoxanthine	TVB-N	Total Viable Nitrogen
HxR	Inosine	TVC	Total Viable Counts
IMP	Inosine-5-Monophosphate	UN	United Nations
ISO	International Standards Organization	VP	Vacuum Packaging
KOH	Potassium Hydroxide	WTPC	Water Vapour Permeability Coefficient
L&H	Long and Hammer agar	WVTR	Water Vapour Transmission Rate
MA	Modified Atmosphere		

1. Introduction

There is an overall growing concern towards sustainability issues, whereas ensuring sustainable consumption and production patterns have become one of the 17 UN Sustainable Development Goals. Balanced and acceptable levels of sustainable development in its three dimensions – economic, social, and environmental – are targeted to be achieved by 2030 in the United Nations. A substantial reduction of food waste at both retail and consumer levels are incorporated into the UN Sustainable Development Goals, along with reduction of waste through recycling and re-use (UN, 2015). The amount of food waste worldwide is estimated to be 1.3 billion tons annually at the retail or consumer level, which corresponds to 1/3 of the yearly global food production. The main function of food packaging is to better protect food products, which is why it may be a viable solution towards reducing food waste in the future. Plastic packaging can also increase a products shelf-life (Wohner, Pauer, Heinrich, & Tacker, 2019).

Today, 26 % of all plastic usage is in application for packaging purposes, and conventional plastic materials often rely on non-renewable resources. They are in many cases nonbiodegradable or not fully recyclable, and often draws on petroleum feedstock with a significant carbon impact (Jefferson, Robert, & Edward, 2009). 90 % of all plastics produced (not just for packaging) derives from petroleum feedstocks (Schmidt, Ximena, Leadley, Potter, & Azapagic, 2019). With the current growth of plastic usage globally, it is estimated to account for 20 % of the total oil consumption and 15 % of the global annual carbon budget by 2050 (Ellen Macarthur Foundation, 2017; Schmidt et al., 2019).

In 2019 Atlantic salmon represented 93.9 % of all produced fish for food consumption in Norway, equivalent to 1,364,044 tons, and had a first-hand value of 68 billion NOK (Statistics Norway, 2020). The salmon industry has, over several decades, been essential to Norway's export economy and will continue to be of significant importance for Norway in the future (Norges sjømatråd, 2021). The innovation project, SeaPack, directed focus towards optimising the usage of plastics in the seafood production industry to increase sustainability, profitability, and reduce environmental impact related to food waste, material consumption, and transport. The project did not focus on replacing plastics derived from petroleum feedstock with biobased options but more on reducing the thickness of plastic films without reducing the shelf-life and

quality. Results from the project lead to a 10 % reduction of plastics used in one of the world's largest producers of salmon, SalMar ASA, which resulted in an annual reduction in plastic consumption of 27 tons (Nofima, 2020). Food packaging alone, provides a more sustainable value chain by limiting food waste, but increased awareness of the environmental impact of petroleum-based packaging has contributed to increased research and developments of biobased and biodegradable alternatives (Lindh, Williams, Olsson, & Wikström, 2016; Nilsen-Nygaard et al., 2021; UN, 2015)

1.1 Scope and Research Objectives

This thesis aimed to compare two biobased and biodegradable plastic materials with different barrier properties (low and high barrier) to a conventional petroleum-based plastic material (PA/PE) to see their effect on quality and shelf-life to fresh salmon fillets during 20 days of storage (4 °C). The goal was to maintain the quality and shelf-life of portioned Atlantic salmon fillets packaged in biobased and biodegradable materials using a petroleum-based material as control.

The objective of this study was to investigate two packaging methods; vacuum packaging (VP) and modified atmosphere (MA) packaging (60 % carbon dioxide (CO₂), 40 % nitrogen (N₂)). Each packaging method was conducted as two separate experiments. Soluble gas stabilisation (SGS, 18 hrs with 100 % CO₂), as a pre-treatment, was used as an experimental factor within each packaging method, to see if it had any further advantages for the fish's quality.

2. Literature Review

2.1 Food Packaging Materials

The primary purpose of food packaging is to contain its product and provide protection during distribution and storage such that the quality maintains (Marsh & Bugusu, 2007). Food packaging has mainly three main functions. The first one being containment, i.e., to keep a product secure from leakage until the packaging is unsealed by the consumer (Cutter, 2002). The second function is mainly directed towards several possible hazards, e.g., microorganisms, oxidation, moisture damage or external physical damage. The last function concerns convenience throughout the value chain (Coles, McDowell, & Kirwan, 2003). It has to be convenient throughout production and transportation, and consumer-friendly in the sense of communication through labelling, easy opening, and suitability for disposal. It can also have the benefits of being easy to recycle or re-use (Fellows, 2017).

There are many different materials used for food packaging, e.g., glass, metal, paper/paperboard, where each material has several subgroups (Marsh & Bugusu, 2007). This thesis purely directs focus on plastic materials.

There are two major categories within plastics: *thermosets* and *thermoplastics* (Alauddin, Choudhury, El Baradie, & Hashmi, 1995). The differences between the two are based on how they react to the application of heat. Thermosets are polymers that solidify and cannot be remoulded after initial forming (Liu, Zhao, & Zhang, 2020), while thermoplastics can be reheated, remoulded, and generally softened upon exposure to heat without causing any chemical changes (Walsh & Kerry, 2012). Conventional plastic materials used for food packaging are in many cases made of thermoplastics due to their several advantages. Thermoplastics can be made into many different shapes and create design flexibility that producers often desire. They are chemically resistant, and therefore an inexpensive product considering the ranges of both physical and optical properties. Many plastic materials are easy to print, tolerable of heat sealing, and can in many cases be formed, filled, and sealed within the same production line (Coles & Kirwan, 2011). These are advantages that many producers of food products take into account. Some of the disadvantages of plastics are the variable permeability on barrier properties such as water vapour, light, and gasses (e.g., O₂, CO₂, and N₂) (Marsh & Bugusu, 2007).

2.1.1 Food Packaging Permeability

Permeation is the process where molecules (such as gas, liquid, or vapour) penetrate a polymeric material (Sangaj & Malshe, 2004). The variation of permeabilities between different plastic materials can be caused by different factors, such as the polymer characteristics of the polymer films or the size of molecules permeating (Lee, 1980). Therefore, it can cause different degrees of permeability to small molecules such as gases, water vapour, and compounds with low molecular weight (e.g., aroma, flavour, etc.) (Siracusa, Blanco, Romani, Tylewicz, & Dalla Rosa, 2012).

Figure 1 illustrates how the different concentrations of molecules (or substances) on each side of a packaging material will undergo a permeation from high concentration to low concentration (Ebnesajjad, 2013). It can be explained by using Henry's law (sorption (p_1, c_1) and desorption (p_2, c_2)) and Fick's law (diffusion), where the rate of permeation correlates with the thickness (l) of the polymer film and the permeant pressure (with $p_1 > p_2$) along with the different concentration of molecules on each side of the film (with $c_1 > c_2$) (Siracusa, 2012).

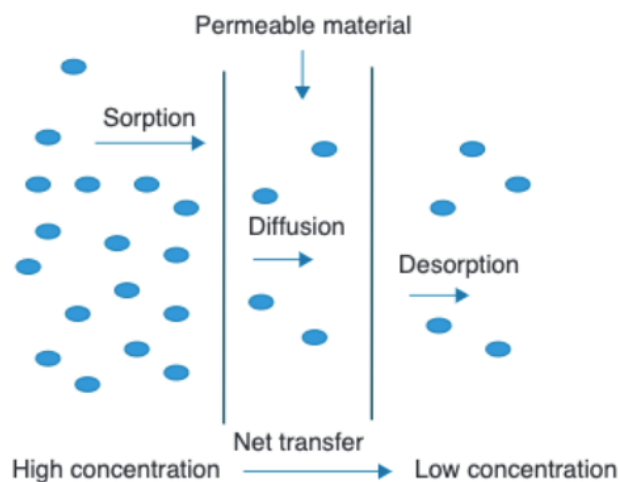


Figure 1: Illustration of molecules permeating through a plastic packaging material (Ebnesajjad, 2013)

Entirely avoiding contamination from the external environment and ensuring a controlled environment inside the packaging is crucial for maintaining both quality and expected shelf-life to the product that requires such conditions (Ebnesajjad, 2013). Permeability is also affected by ambient environmental factors such as temperature, humidity and pressure (Siracusa et al., 2012).

Three of the most important barrier properties of polymer films used for food packaging are the following:

Oxygen Transmission Rate (OTR)

The oxygen barrier of a plastic film is essential for a product's preservation in many cases and is vital whether O₂ has to be kept inside the packaging or if the goal is to keep O₂ from permeating into the packaging (Massey, 2003). Oxygen transmission rate (OTR) refers to the rate of oxygen permeating through a polymer, and is often provided as targeted values on basis of standard tests (Abdellatif & Welt, 2013). The process of permeation through packaging are often described by Fick's law, and the equation of OTR may be expressed as:

$$\text{OTR} = P_{\text{eff}} \cdot A \cdot \frac{\Delta p}{l} \quad (\text{Equation 1})$$

Where P_{eff} is the effective coefficient of permeability, A is the unit area available for O₂ transfer, ΔP ($p_1 - p_2$) is the difference between the oxygen partial pressure (p_1) on the inside and p_2 is equal to zero on the detector side, and l is the thickness of the plastic film. The OTR value is often given as O₂ cc/day or cc/m²s (Fellows, 2017; Poças, Ferreira, Pereira, & Hogg, 2010; Siracusa, 2012).

Carbon Dioxide Transmission Rate (CO₂TR)

The carbon dioxide (CO₂) barrier plays a significant role in food packaging that requires CO₂ holding capacity to maintain or extend a product's shelf-life. Carbon dioxide transmission rate (CO₂TR) refers to the rate of transmission through a polymer (Khan et al., 2013). It can be described with the same principles as the OTR value (Equation 1) (Murmu & Mishra, 2017).

Water Vapour Transmission Rate (WVTR)

The water vapour transmission rate (WVTR) refers to the rate of water vapour which permeates through a polymer, and is often a critical parameter for flexible organic packaging (Nakano, Yanase, Nagahama, Yoshida, & Shimada, 2016). There are several methods to measure WVTR, and methods can vary among what kind of polymer film that is evaluated (Nakano et al., 2016). It indicates the amount of water vapour permeating per unit area of the packaging material over time. The WVTR is often expressed in cc/m²s (Siracusa, 2012).

2.1.2 Plastics with High Barrier Properties

As stated above (see 2.1.1), the amount of molecules that penetrate through a plastic material often depends on the characteristics of the polymer film, among other factors. The chemical structure, degree of crystallinity, or thermal properties can affect the degree of permeation. Single-layer plastic films can often be reasonably permeable, and one solution to prohibit permeation is to design multi-layer films to amplify the resistance (Ebnesajjad, 2013). Combining different polymers with different characteristics can result in a product with high barrier properties to water vapour, gas, and oxygen. The challenging part is to create a product that obtains all the positive characteristics from each polymer (Lagaron, 2011).

2.1.3 Polyamide/Polyethylene (PA/PE)

Flexible multilayer packaging can meet diverse requirements for food packaging by combining different materials with different barrier properties (Wagner, 2016). Polyamide (PA) is a polymer that reduces oxygen permeability and has mechanical strength, while polyethylene (PE) has sealable properties and provides low water vapour permeability. PE is the most used thermoplastic in flexible packaging applications. A combination of the two can result in a vacuum pouch and thermoformed films with good barrier properties and mechanical stability, and are ideal for food packaging (Pauer, Tacker, Gabriel, & Krauter, 2020). A study done by Larsen (2004) showed measurements of OTR in different types of packaging materials at different temperatures, where a PA/PE vacuum pouch (Allfo Verpackung, Waltenhofe, Germany) with 90 μm thickness had an OTR value of 0.3 ± 0.02 ml $\text{O}_2/\text{pkg}/24\text{hrs}$ at 6 °C. Both PA and PE are originally derived from petroleum resources, but has the ability to be produced as biobased non-biodegradable materials (Rahman & Bhoi, 2021).

Ethylene Vinyl Alcohol (EVOH)

Ethylene vinyl alcohol (EVOH) is a copolymer that can be used as a tie layer between two layers of polymers, such as PA and PE. The material is generally highly crystalline, depending on the levels of ethylene content. A typical resin within EVOH is a product named EVAL™, a resin which is widely used in the food packaging industry as a barrier layer due to its ability to offer good barrier properties (Massey, 2003). Based on the level of ethylene content in the polymer, it shows good resistance of oils and organic solvents, making it suitable for packaging food containing high lipid levels. It also offers excellent gas barrier properties and good

preservation of aroma within the package (Ebnesajjad, 2013). Looking at a circular economic perspective, a plastic material (e.g., PA/PE) that includes a mixture with EVOH is less desirable than a mono-material (e.g., PET) when it comes to producing a higher-value recycled product (Schmidt et al., 2019).

2.1.4 Polyethylene Terephthalate (PET)

The prominent member of the thermoplastic polyester family is considered to be polyethylene terephthalate (PET). It is widespread in commercial and industrial applications and is mainly obtained from chemical materials found in petroleum feedstock. PET has good barriers for water vapour and gas, and is manufactured in flexible films, fibres, and containers, which can be formed into both simple or complex shapes (Barber, 2017). During variations of temperature and humidity, the PET material undergoes little shrinkage, making it a stable material to use in the industry. Another form of PET is crystalline PET (CPET) which is a non-transparent material that can be made into semirigid trays to contain a product (Fellows, 2017). According to Abel, Rotabakk, and Lerfall (2020) CPET trays (300mL, C2125-1B, Færch Plast, Holstebro, Denmark) had an OTR value of $66-78 \text{ cm}^3 \times 25 \mu\text{m} \times \text{m}^2 \times 24 \text{ h}^1 \times \text{bar}^1$ at 23 °C.

Along with the growing concern for developing more sustainable alternatives, PET has caused a problem due to it being non-biodegradable (Andreeßen & Steinbüchel, 2019). The material is, as stated above, derived from petroleum resources, and therefore has no renewable or biobased origin. Nevertheless, PET can be recycled, even though its degradation and possibility of contamination are an issue. PET's degradation products in a recycled PET (rPET) can in fact contaminate a food product. Substances that can be contaminated into recycled PET are acetic acid, fragments of colour from previous PET products, acetaldehyde, and other contaminants (e.g., detergents, fuel, pesticides, etc.). Therefore, specific requirements for minimum levels for plastic recycling exist to avoid any contamination risks, where the food industry additionally have their own requirements to ensure food safety based on packaging materials (Barber, 2017).

2.1.5 Biobased and Biodegradable Packaging Material

The massive consumption of packaging materials derived from petroleum feedstock has over the years contributed to environmental challenges, such as draining of natural resources, global warming, and pollution (Schmidt et al., 2019). The increasing environmental awareness inflicted upon packaging materials derived from petroleum feedstock has increased the focus

on biobased polymers and biodegradable packaging materials. It has come to show that biodegradable polymers derived from renewable resources can both replace and reduce the usage of petroleum-based plastic packaging, leading towards less environmental impact (Song, Xiao, & Zhao, 2014).

Bioplastics are either biobased, biodegradable, or both. According to the European Bioplastic Organization (2018), a biobased material can be only partly or entirely derived from biomass of renewable resources (e.g., cassava root, corn starch, vegetable oils, etc.). The resources used in biobased plastic can derive from biogenic residues and waste, and should not compromise resources that can be utilized directly as food (Weiss et al., 2012). On the other hand, biodegradable materials are made of ingredients that can undergo a chemical process and be metabolized by naturally occurring microorganisms from the environment, that can further convert the material into natural substances (such as water, CO₂, and regular compost) (Lambert & Wagner, 2017). Biodegradable material does not rely on resources as much as it relies on its chemical structure. Fully petroleum-based plastic material can in fact be biodegradable, while a fully biobased plastic material can be non-biodegradable. The fully petroleum-based plastic that can biodegrade will usually take a very long time and contribute to a higher release of CO₂ than biobased and biodegradable plastic would. The terms are therefore important to distinguish from each other (Ebnesajjad, 2013).

While biodegradation happens in the action of enzymes and/or chemical deterioration that correlates with living microorganisms, is composting (also called organic recycling) related to a more enhanced biodegradation (Nilsen-Nygaard et al., 2021). Composting happens in a more controlled environment with managed conditions, such as temperature, humidity, microorganisms, and often with a timeframe. The compostable plastic material, along with being biodegradable, will have a resulting output of a material (compost) that can be utilized further into soil amendment products and give nutrients to soil (Napper & Thompson, 2019).

To be able to label a material as biodegradable or compostable, there are several specification standards and requirements that need to be fulfilled. The European Union has provided standards such as EN13432 ‘Requirements for Packaging Recoverable through Composting and Biodegradation’, and International Standards Organization (ISO) has its own standard; ISO 17088 ‘Specification for Compostable Plastics’ (European Union, 2000; ISO, 2012). There are several standards worldwide concerning composting and biodegradation that are either suitable

for the industrial composter, home composter, or laboratory-based composting (Napper & Thompson, 2019). There are still main principles and requirements for biodegradation under composting conditions among all these standards that are similar (Song, Murphy, Narayan, & Davies, 2009);

- I. The material needs to be able to convert into CO₂, biomass, or water through assimilation by microorganisms.
- II. 90 % of the material's carbon converts into CO₂ to sets the biodegradation's statistical variability to ± 10 %.
- III. The material has the same biodegradation rate as natural materials (e.g., grass, leaves, food fragments).
- IV. Timeframe for biodegradation under compost is 180 days or less.

Cassava Root, Corn Derivatives and PolyButylene Adipate-co-Terephthalate (PBAT)

Some of the most common renewable starches for the production of biodegradable films are cassava, corn (maize), potatoes and wheat (Rodrigues et al., 2021). Corn starch contributed to approximately 77 % of the total starch production, while cassava is second in line with approximately 12 % (Shevkani, Singh, Bajaj, & Kaur, 2017). The latter has been increasingly common due to its renewability, biodegradability, and wide accessibility contributing to its low cost (Gutiérrez, Tapia, Pérez, & Famá, 2015; Segura & Sira, 2003). Even though cassava has proven to have rapid degradability along with creating film flexibility and transparency, it also has some limitations to its mechanical properties and high permeability to water vapour (Leal et al., 2019). An alternative way to meet those limitations has been to blend the starch with a co-polymer that would provide such qualities.

Polybutylene adipate-*co*-terephthalate (PBAT) is a fully biodegradable aromatic-aliphatic copolyester based on petroleum feedstock (Ferreira, Cividanes, Gouveia, & Lona, 2019), which degrades within weeks under the right circumstances. Cardoso et al. (2017) studied how PBAT films would preserve fish fillets during storage and concluded that the film presents suitable characteristics for application as food packaging because of its thermal, mechanical and water vapour barrier properties. Leal et al. (2019) examined a flexible film blended with cassava starch and PBAT and concluded that it had adequate properties for packaging fresh mango if blended correctly with a compatibilizer. Since the cassava starch has hydrophilic characteristics and the PBAT has hydrophobic, a blend of them alone will result in poor interfacial adhesion.

A compatibilizer, such as citric or lactic acid, is required to achieve better interfacial adhesion. One of the largest renewable sources for lactic acid, which also increases biodegradability, are corn derivatives (Jayathilaka, Ariyadasa, & Egodage, 2020). The large global production volume of corn results in a large amount of corn waste. Utilizing corn waste as a polymeric material is environmental friendly in terms of contributing to corn's life cycle (Xu, Qiao, & Sun, 2020).

Cellulose Film and Biobased PolyButylene Succinate (BioPBS)

Cellulose is one of the most abundant natural polymers on earth, and is considered to be a biodegradable plastic because of its long-chain aliphatic acid esters (Joly, Granet, Branland, Verneuil, & Krausz, 2005). It can be biosynthesized by several microorganisms and is an environmental friendly product because of its short biodegrading period (Othman, Adam, & Mat Yasin, 2021). Cellulose provides enhanced barrier properties because of its crystalline fibres, and has proven to improve mechanical and water vapour barriers for chitosan films if only added 15 % of cellulose nanofibres into the blend (Pandey, Takagi, Nakagaito, & Kim, 2015).

Polybutylene succinate (PBS) is a thermoplastic polyester with good biodegradability. It can be produced from either petroleum resources or renewable biobased resources (Gowman, Wang, Rodriguez-Urbe, Mohanty, & Misra, 2018). Biobased PBS (BioPBS) has been proven to become a promising and more sustainable alternative to petroleum-based PBS since it can be produced with a range between 54-100 % biobased resources through synthesizing succinic acid and butanediol. It has similar mechanical properties to polyethylene, and has good thermal and chemical resistance (Tan, Bi, Emery, & Sobkowicz, 2017).

2.2 Atlantic Salmon

Atlantic salmon (*Salmo salar* L.) is a pelagic species that is widespread in the Northeast Atlantic ocean (Figure 2) (Hjermann, 2020; Vøllestad, 2019). The chemical composition of the fish is ~20g protein, ~11g lipid, <0.1g carbohydrates and ~67g water (Holland, Brown, & Buss, 2012). The composition can vary due to age, size, season, swimming activity and environmental condition (Dunajski, 1979; Mørkøre & Rørvik, 2001; Shearer, 1994; Shearer et al., 1994).

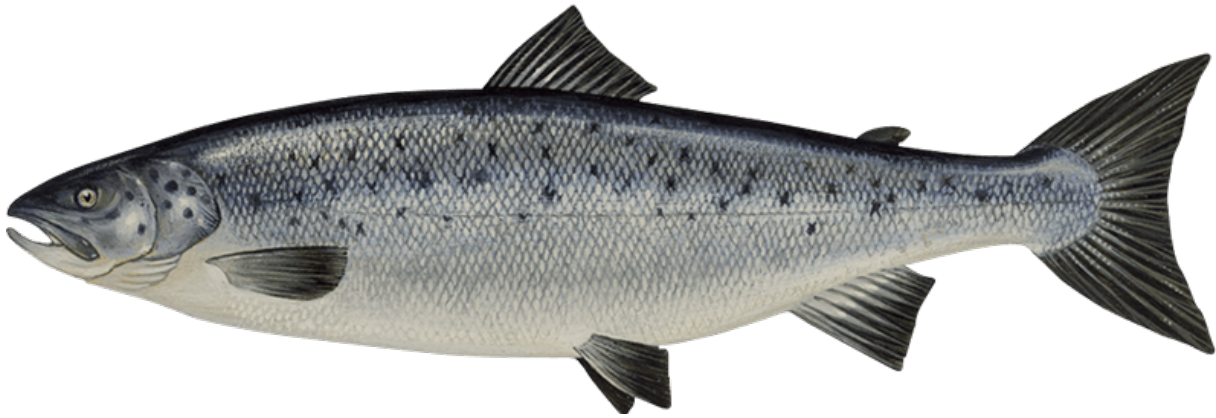


Figure 2: Illustration of Atlantic salmon (Young's, n.d.)

2.3 Spoilage of Atlantic Salmon

Food spoilage is considered a change or process that results in an undesirable or unacceptable product for human consumption. Seafood, in general, deteriorates rather quickly, and Atlantic salmon is considered to be a perishable product due to its nearly neutral pH level ($\text{pH} > 6$), high water activity ($a_w > 0.98$), high lipid level (high in polyunsaturated fatty acids) and available nutrient level (Socaciu, Semeniuc, & Vodnar, 2018). The shelf-life has been observed to be 20 days if stored whole in ice, while storage at chilled temperature of 2-4 °C decreases the shelf-life down to approximately 14 days (Sivertsvik, Rosnes, & Kleiberg, 2003). Quality degradation and deterioration of salmon occur mainly through three processes; enzymatic degradation, microbial deterioration, and the chemical oxidation of lipids (Boziaris, 2014).

2.3.1 Enzymatic Degradation

Immediately following death, the supply of oxygen to the muscles will stop due to the absence of blood circulation, leading to the process of *rigor mortis* to begin. *Rigor mortis* is when the fish undergoes biochemical changes that result in loss of flexibility due to stiffening of the muscles (Hong, Regenstein, & Luo, 2017). This process is highly related to pre-mortem stress (Berg, Erikson, & Nordtvedt, 1997; Mørkøre, Mazo, Tahirovic, & Einen, 2008) with an onset normally starting within 24 hours after death. The endogenous enzymes present will cause an autolysis.

Adenosine triphosphate and its degradation products

The degradation of nucleotides and their enzymes results from the many changes that occur within hours after death (Donaldson & Lamont, 2013). The natural aerobic generation of

adenosine triphosphate (ATP) will stop as the remaining oxygen level drops quickly (Wilson, Erecińska, Drown, & Silver, 1979). ATP can be seen as the “energy currency” of the cell, and the fish’s muscles use the majority of this energy to perform mechanical work and synthesize urea, proteins, and other metabolic processes. Nevertheless, the muscles will still try to keep the ATP level steady without oxygen, causing anaerobic glycolysis (Huss, 1995).

Muscle glycogen breaks down after the fish is slaughtered, causing a short period of ATP production. Without any glycogen left in the muscles, the remaining ATP will subsequently undergo a series of biochemical reactions and degradation (Boziaris, 2014), as shown in Figure 3. ATP will eventually start its degradation process into adenosine diphosphate (ADP) followed by adenosine monophosphate (AMP) (Wilson et al., 1979). These reactions will take place rather rapidly. As the concentration of AMP increases, it will consequently deaminate into inosine-5-monophosphate (IMP). IMP can be associated with a pleasant savoury taste of umami and is commercially used throughout the food industry as a flavour enhancer (Bagnasco et al., 2014). The deterioration of the fish starts when IMP is hydrolysed by autolytic enzymes and produces inosine (HxR) and hypoxanthine (Hx). Hx will further transform into xanthine and uric acid products, which will develop spoilage microflora and deteriorate the fish (Hong et al., 2017; Karim et al., 2019).

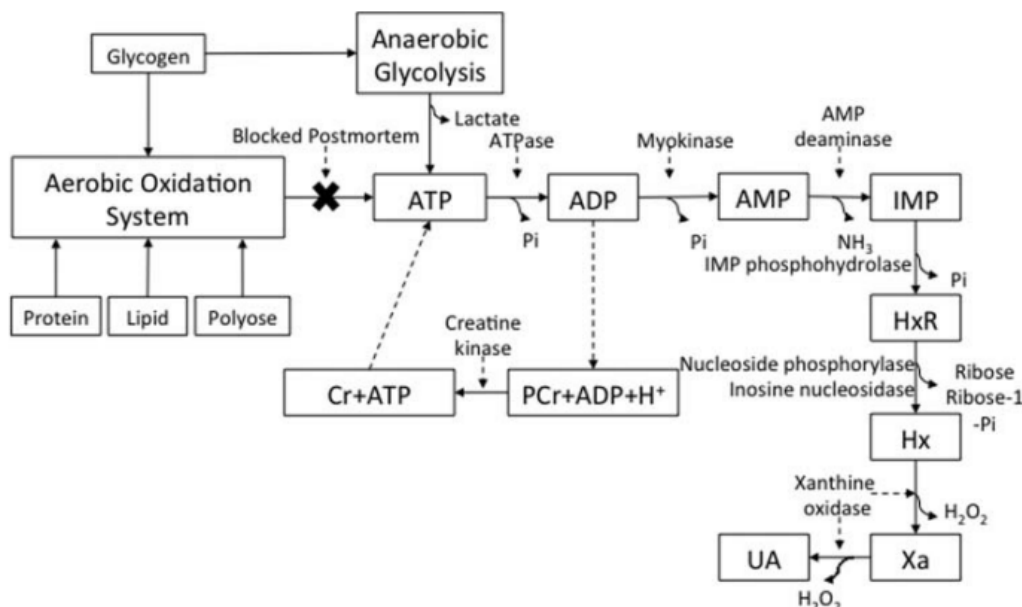


Figure 3: Detailed ATP degradation process in post-mortem fish muscle (Hong et al., 2017)

K-value and H-value

The overall freshness of fish can be assessed by determining the K-value (%) of the fish, an index based on the ratio of HxR and Hx, divided by the total quantity of ATP and its degradation products. Since the production of HxR and Hx align well with the decrease of the freshness, the K-value will have a higher value according to the decrease of freshness (Simpson et al., 2012). The K-value is known to be depending on variations, e.g., season, species, degree of handling conditions, and capture and slaughtering methods. Therefore, it can be a disadvantage to use the K-value as the index of freshness in some cases (Olafsdóttir et al., 1997). The formula of the K-value is expressed as :

$$K - value(\%) = \frac{(HxR + Hx)}{(ATP + ADP + AMP + IMP + HxR + Hx)} \times 100 \quad (\text{Equation 2})$$

The accumulation of HxR has been shown to increase very rapidly for some species of fish during ATP degradation, that K-value might not be the best indicator of freshness (Park & Kim, 1999). Due to this, and the fact that the K-value is so dependent on different variations, the H-value is an alternative that only considers IMP, HxR and Hx. The H-value can, in some cases, be a better indicator of the degree of flavour development since IMP contributes to flavours associated with freshness, while Hx contributes to a more bitter and off-flavour attribute (Abel, 2021). It has also been reported to correspond better with sensory assessments (Rzepka, Özogul, Surówka, & Michalczyk, 2013). The formula of the H-value is expressed as (Luong, Male, Masson, & Nguyen, 1992):

$$H - value(\%) = \frac{(Hx)}{(IMP + HxR + Hx)} \times 100 \quad (\text{Equation 3})$$

2.3.2 Microbial Deterioration

The metabolic activity of spoilage microorganisms is one of the largest contributors for deterioration and loss in quality (Ashie, Smith, Simpson, & Haard, 1996). Salmon muscle's high level of non-protein nitrogenous (NPN) compounds and pH level being nearly neutral makes a suitable environment allowing fast growth of microorganisms and specific spoilage organisms (SSOs) (Bozaris, 2014). The spoilage biota refers to the total amount of bacteria present when the fish spoils, while the spoilage bacteria refers to SSOs that produces off-flavours and off-odours, leading to spoilage (Huss, 1995). The most dominant SSO for deterioration of fish under aerobic conditions are *Pseudomonas* spp., while *Photobacterium*

phosphoreum and *Shewanella putrefaciens* have more dominance under anaerobic conditions (e.g., during storage in packaging with little or no oxygen available) (Saraiva, Vasconcelos, & de Almeida, 2017). As seen in Figure 4, the spoilage biota (total count) is higher than the SSO level, to the point where the sensory rejection hits. The figure is only an illustration, saying nothing about fish species or its duration of storage since those factors will always be affected by different parameters.

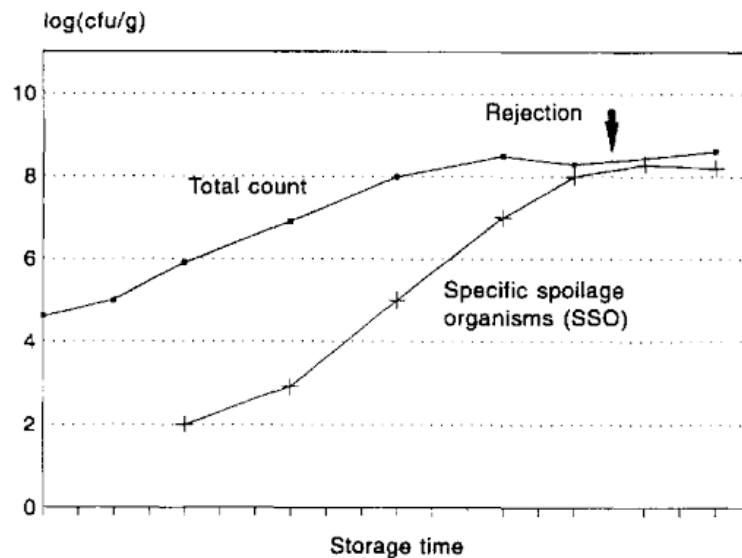


Figure 4: Illustration of the relation between total counts and specific spoilage bacteria during storage (Huss, 1995)

Professional Food Microbiology group (PFMG) of the Institute of Food Science and Technology (IFST) has set microbiological criteria for raw fish (including Atlantic salmon) to be below 10^6 CFU/g of total viable counts (TVC) or aerobic plate counts (APC) immediately after production under good manufacturing practice (GMP). Meanwhile, the maximum values throughout the shelf-life are acceptable up to 10^7 CFU/g (Bell, 1999). Time of sensory rejection has no exact link to a specific bacterial concentration, but several studies have reported that it happens when the levels are between 10^6 - 10^8 CFU/g (Dalgaard, Gram, & Huss, 1993; Dalgaard, Mejlholm, & Huss, 1997; Nuin et al., 2008).

2.3.3 Chemical Oxidation of Lipids

Oxidation of lipids and reactions caused by the fish's own enzyme activity can lead to trimethylamine (TMA) and total volatile nitrogen (TVB-N) production, which are indicators of microbial spoilage of fish products (DeWitt & Oliveira, 2016; Sivertsvik, Jeksrud, & Rosnes, 2002). TVB-N is a physiochemical index which can indicate a degree of spoilage towards the

end of the fish's shelf-life, since the TVB-N production increases with storage time rapidly towards rejection (Sallam, 2007).

2.3.4 Drip Loss

Another parameter of commercial concern to the quality is the subsequent drip loss during processing, storage and/or thawing. Drip loss is the free moisture that denotes the liquid that contains both water and soluble nutrients and flavour compounds (Duun, 2008). The substances leak from the fish's cells during processes such as storage. It is also visually unappealing for the consumer, as well as the product's texture can result in a drier consistency. It correlates to the fish's water holding capacity (WHC), which defines the muscle's ability to hold water (Chan et al., 2021). Drip loss can occur due to reduced WHC, which can happen due to alterations such as myosin denaturation, increased extracellular space or shrinkage of myofibril lattice (Kaale & Eikevik, 2015). The WHC is moreover affected by the state of *rigor mortis* and time of filleting resulting in significant lower drip loss of pre rigor filleted Atlantic salmon (Rotabakk, Melberg, & Lerfall, 2018).

2.4 Packaging Methods

Food packaging aims to enclose the product to protect it from tampering or contamination from physical, biological and chemical sources and needs to deliver safe products in sound condition to the final consumer (Cutter, 2002). Packaging is one of the most essential parts of food production and one of the most dynamic sectors in food processing (Fellows, 2017). Minimally processed foods, such as fresh salmon, has specific packaging requirements to be able to maintain a fresh product throughout its estimated shelf-life. Packaging methods such as VP, MA packaging, edible coatings and active packaging are relevant methods for minimally processed foods since it protects the product from the outer environment as well as provides a response to changes within the package (Fellows, 2017; Wani, Singh, Pant, & Langowski, 2015).

2.4.1 Vacuum Packaging

VP is commonly used for packaging of fatty fishes, e.g., salmon. The technique removes the natural atmosphere and headspace within the package after the product is placed inside the pouch (Parra et al., 2012). The most essential part of this process is the extraction of O₂ from the headspace to prevent oxidation reactions, such as lipid oxidation, loss of pigments or

specific vitamins and TMA production, especially if the films have high O₂ barriers. Next to oxidation reactions, VP also prevents deterioration by aerobic microorganisms. An additional advantage is the reduction of volume for distribution (Wani et al., 2015).

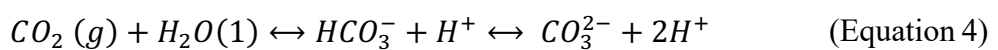
2.4.2 Modified Atmosphere Packaging

MA packaging is also commonly used for packaging of fresh fish, and the method involves replacing the regular atmosphere (approx. fractions by volume: 20,9 % O₂, 78 % N₂ and 0,03 % CO₂ (Brimblecombe, 1986)) with a MA before sealing the package. It is normally conducted by placing the product in a tray that has a top film sealed over it, and the headspace within the package is left with MA. The applied gas has different desirable compositions depending on the product's composition inside the package (Fellows, 2017). The three preliminary gasses used in MA packaging are O₂, N₂ and CO₂, and each of the three gasses influence the food quality and shelf-life in different ways (Wani et al., 2015). MA packaging with a headspace gas mixture at 60 % CO₂/ 40 % N₂ has been proved to extend the shelf-life of fresh salmon fillets by seven days compared to vacuum-packed salmon (Hansen et al., 2009).

To avoid oxidation and growth of psychrotrophic bacteria that can cause food spoilage for fishery products, the O₂ level in MA packaging is usually set to 0 % of the total gas mixture. The CO₂-enriched atmosphere can inhibit the most common aerobic spoilage microorganisms and postpone lipid oxidation, which is common in more fatty fish. CO₂ is seen as the most important gas because of its bacteriostatic and fungistatic properties. However, the success of MA packaging is determined by the amount of dissolved CO₂ into the product (Sivertsvik et al., 2002). For CO₂ to inhibit bacterial growth, a suitable amount of dissolved CO₂ is required in the product, and therefore determines the overall effectiveness of MA packaging. Sivertsvik, Rosnes, and Jeksrud (2004) performed a study showing that the partial pressure of CO₂ and the gas volume to product volume ratio (g/p) can be used to determine the amount of dissolved CO₂. A low g/p , caused by the high amount of dissolved CO₂ into the product, could result in package collapse if the material is flexible (Rotabakk, Lekang, & Sivertsvik, 2007). The pressure decreases because dissolved CO₂ has less volume than CO₂ gas. For fishery products, N₂ is usually a part of the MA's gas mixture to avoid the package to collapse by lowering the CO₂ partial pressure. N₂ is an inert gas that has significantly less solubility than CO₂. N₂ also helps to prevent oxidation and development of off flavours (Erkan, Özden, Alakavuk, Yildirim, & İnuğur, 2005; Fernández, Aspé, & Roedel, 2010).

2.4.3 Soluble Gas Stabilisation

SGS is an alternative method to enhance the MA inside the packaging to obtain the quality and possibly extend the shelf-life of the fish (Sivertsvik, 2000; Sivertsvik & Birkeland, 2006). As mentioned above, the effectiveness of MA packaging is mainly governed by the amount of available CO₂ dissolved into the product. SGS is a method based on saturating the fish in 100 % CO₂ at chilled temperatures and elevated pressure (≥ 2 atm) prior to packaging, resulting in muscle absorbing CO₂ (Mendes, Pestana, & Gonçalves, 2008). This is done according to the following chemical reaction:



Sivertsvik, Jeksrud, Vågane, and Rosnes (2004) performed a study based on Henry's law showing that a product will after 3 hours in 100 % CO₂ lead to the same amount of dissolved CO₂ in the products as it would if the product were stored for 48 hours at 50 % CO₂. This suggests that the technique can also be successful with a package that has a smaller *g/p*, and the saturation of CO₂ can also be satisfied under VP (Mendes & Gonçalves, 2008).

2.5 Methodological Theory

2.5.1 LAB Colour Space

The CIE L*a*b* colour space shows the whole spectrum of colours and nuances, and is illustrated with a quantitative relationship between three axes (Figure 5). The vertical axis represents L, which has a value that ranks from 0 (black) to 100 (white), indicating the lightness. The a* and b* axes are chromaticity coordinates and represent different colour channels (Pecho, Ghinea, Alessandretti, Pérez, & Della Bona, 2015). The a* value indicates components of colours from -128 (green) to +128 (red), and b* value indicates the colours -128 (blue) to +128 (yellow). The distance from the centre of the diagram, which is colour neutral, represents the chroma (C*). C* can be described as the saturation or intensity of a colour (Roy Choudhury & Naskar, 2019). Hue represents the angular position around the diagram and indicates the degree to which a stimulus can be described (Ly, Dyer, Feig, Chien, & Del Bino, 2020; Murali & Govindan, 2013).

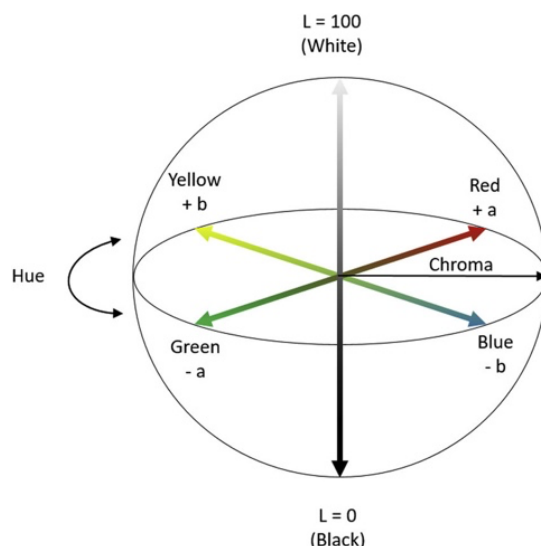


Figure 5 Pictorial representation of the CIE $L^*a^*b^*$ colour space diagram. L value represent lightness (0-100), a^* represent green to red (-128-128) and b^* represent blue to yellow (-128-128) (Ly et al., 2020)

2.5.2 Texture

Textural properties for fresh salmon is one of the main parameters to determine quality by analysing the firmness (Dunajski, 1979). A stored salmon fillet may have acceptable colour, taste and odour, and at the same time be too soft for general acceptance. The muscle tissue of fish will undergo tenderisation during post mortem changes due to the degradation of collagen fibrils leading myotomes to separate from myocommata. Lactic acid and pH reduction during post mortem changes can induce leakage of proteolytic enzymes, leading collagen cross-links to break (Veland & Torrissen, 1999). Decreased connective tissue and weakened cross-links between collagen molecules will cause gaping and result in a softer texture (Morzel, Heapes, Reville, & Arendt, 2000).

Among the different methods to determine textural properties, objective measurements using mechanical equipment can lead to a more precise result eliminating human error rather than sensory evaluations (Sigurgisladottir et al., 1999). To distinguish between the same raw material and storage day, which only differ between different packaging material types, the exact force (N) needed to break the surface (breaking force), the resistance force of the muscle, and overall fillet firmness will be easier to detect with mechanical equipment.

Several factors can influence the textural properties of fresh salmon, e.g., age, size, fat content, proteases and seasonal variation. Chemical composition and physical structure will affect the texture, and there may be a natural variation among salmon harvested at the same time and

among the different seasons of harvesting (Jonsson, Sigurgisladottir, Hafsteinsson, & Kristbergsson, 2001). According to a study by Espe et al. (2004), farmed salmon harvested in February and stored 14 days on ice had connective tissue with more soluble collagen and less insoluble collagen than salmon harvested in June. The gaping score was higher, and the fish was softer among fish harvested in February, and there was an interaction between more insoluble collagen and less gaping among fish harvested in June.

2.5.3 High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a widely known technique used to separate nucleotides and their derivatives. It separates a liquid sample (analyte) based on its distribution between a stationary and a mobile phase. The principle of the system is that the analyte (depending on its chemical structure) will separate while it passes the stationary phase (Özogul, Taylor, Quantick, & Özogul, 2000). Based on the components of the sample and the packing material of the stationary phase, the separated and individual components can be measured and defined since the components will pass the stationary phase at different speed and time. A UV detection unit is used to recognize the components leaving the stationary phase, where the signal measurements are transferred to PC software which generates a chromatogram. The sample will be mixed with the mobile phase through an injection valve and transported by a pump and pressure throughout the system until the sample has gone through the stationary phase (Böttcher, Margraf, & Monks, n.d.).

The stationary phase is originally meant to be a polar packing material of the column, while the mobile phase is an eluent that can differ depending on the desired outcome (Xue et al., 2009). A reverse-phase column (non-polar stationary phase) is often used along with a mobile phase of phosphate buffers utilizing ion-pairing methods to separate individual components in a sequence (Özogul et al., 2000).

ATP and its degradation products can be measured and analysed by performing an HPLC analysis. These products will undergo a separation using a reverse-phase column, a non-polar stationary phase, because of their ease of use and high selectivity. The polar components of the liquid sample will therefore drain from the column first, and subsequently the non-polar components (Bijttebier et al., 2014).

3. Materials and Methods

Regarding this MSc thesis, a two-part study was conducted to establish how biobased and biodegradable packaging materials would affect the quality and shelf-life of Atlantic salmon fillets, by investigating two packaging methods and SGS treatment as an experimental factor. The experiments were carried out at the Department of Biotechnology and Food Science (NTNU) from November 2020 through March 2021.

3.1 Raw Material and Experimental Design

Figure 6 illustrates the timeline of both experiments, including the different distribution chain for each raw material. Atlantic salmon used in experiment one was harvested, slaughtered and gutted by Lerøy AS November 26th, and distributed by Slakteriet AS. The first experiment started November 26th and lasted until December 22nd.

Fresh fillets of Atlantic salmon from Domstein Sjømat AS were used for the second experiment. The raw material was harvested and slaughtered March 1st, packed March 5th in a Styrofoam box with freezer elements with an expiration date set to March 16th (Figure 6). Due to delays on the supplier's side, the raw material did not arrive until March 10th, being nine days post mortem. Nevertheless, there was no opportunity of postponing the experiment due to limited time. The second experiment therefore began on March 10th and lasted until March 31th.

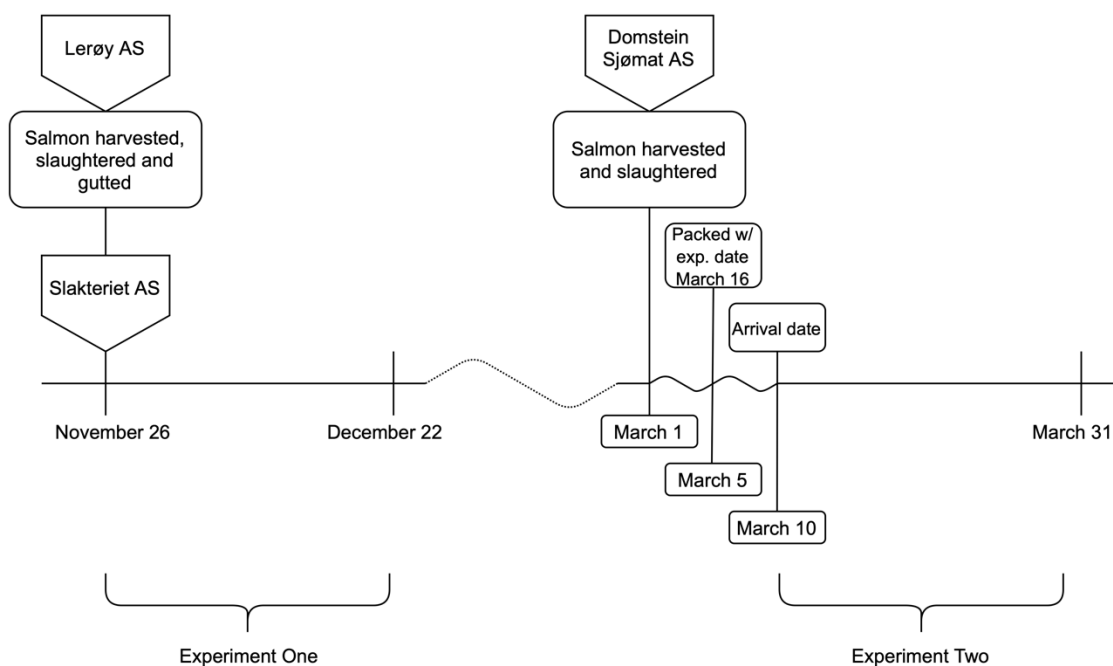


Figure 6: Timeline of both experiments, including the raw material's distribution chain

3.1.1 Experiment One – Vacuum Packaging Combined with Soluble Gas Stabilisation

Figure 7 shows an illustration of the experimental design of experiment one, the first part of the study which was conducted. Raw material (n=6) from day 0 was analysed for drip loss, pH, colour, microbial and biochemical analysis. The remaining raw material was further divided into two groups where one half (three sub-groups) had no pre-treatment before packaging (n=54), while the other half (three sub-groups) were treated with SGS before being re-packed (n=54). The first experiment concerned only one type of packaging method; VP. There were three different packaging materials used in this experiment, one being made of PA/PE (described in 3.1.3) and two biobased materials with a low and high barrier (described in 3.1.3). The duration of storage was 20 days with a storage temperature of 4 °C. Every analysis was performed on sampling day 0, 10 and 20, but microbial and biochemical analyses were additionally performed on sampling day 5 and 15. Sampling day 15 also included pH measurements.

SGS-treatment

SGS samples were placed on 5 trays (C2325-1C, Færch Plast, Holstebro, Denmark) in batches (n=11 in four trays and n=10 on the fifth tray) in high barrier vacuum pouches made of PA/PE (425x650 mm, Maske AS, Trondheim, Norway). The headspace was filled with 100 % CO₂ using a chamber machine (Webomatic SuperMax s3000, Webomatic, Bochum, Germany). The gas was injected by using a gas mixer (MAP Mix 9000, Dansensor, Ringsted, Denmark). The 5 trays were stored for 18 hours with a temperature of 4 °C.

Vacuum packaging

Each sample (50±5g) were placed singularly into their respective vacuum pouches of the assigned material, and the VP was carried out using the same chamber machine as for SGS-treatment. The vacuum pressure was set to 50 mbar. The SGS-treated samples were re-packed into regular VP the day after, 18 hours after the initial experiment start. The sealed packages were stored in a cold room of 4 °C.

The three sub-groups in experiment one without SGS-treatment will later be referred to as:

- **PA/PE vacuum** – samples stored in VP with PA/PE material
- **BioLB vacuum** – samples stored in VP with biobased material (low barrier)
- **BioHB vacuum** – samples stored in VP with biobased material (high barrier)

The three groups in experiment one without SGS-treatment will later be referred to as:

- **PA/PE SGS+vacuum** – samples stored in VP with PA/PE material
- **BioLB SGS+vacuum** – samples stored in VP with biobased material (low barrier)
- **BioHB SGS+vacuum** – samples stored in VP with biobased material (high barrier)

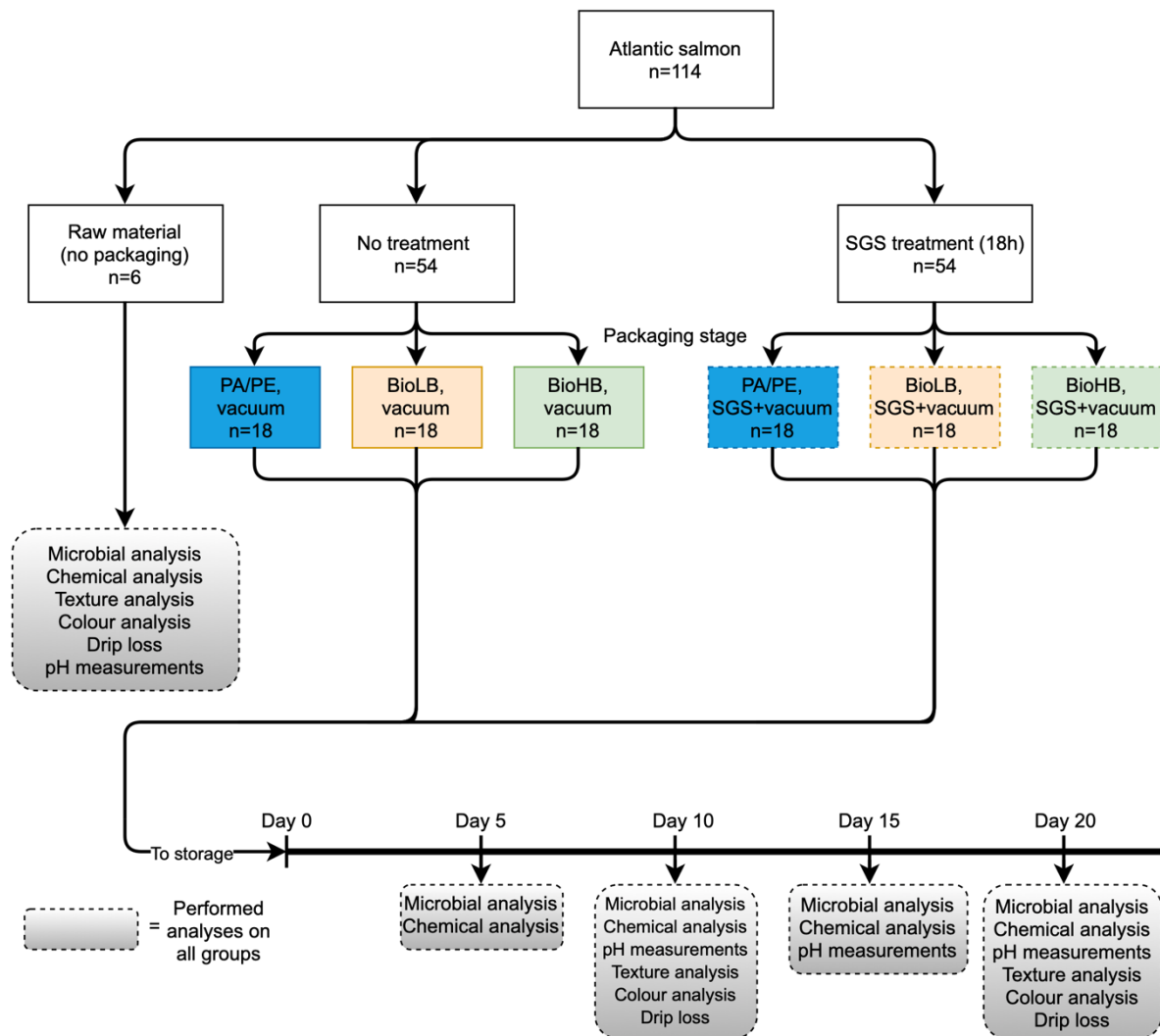


Figure 7: Illustration of the vacuum experimental design. Raw material (n=6) was analysed day 0, all six groups (n=3 from each group each sampling day) at day 5, 10, 15 and 20 for microbial, biochemical analysis, and pH (except for day 5). At sampling day 10 and 20 all six groups (n=3) were additionally analysed for texture and colour. Note that the SGS-treated group was re-packed after 18 hrs, and had one day delay.

3.1.2 Experiment Two – Modified Atmosphere Packaging Combined with Soluble Gas Stabilisation

Figure 8 shows an illustration of the experimental design for experiment two, the second part of the study that was conducted. Raw material (n=6) from sampling day 0 were analysed for drip loss, pH measurements, colour and microbial analysis. Biochemical analysis was not performed during this experiment due to limited time. The remaining raw material was further divided into two groups where one half had no pre-treatment before packaging (n=54), while the other half were treated with SGS for 18 hours before being re-packed (n=54). The second experiment concerned one type of packaging method; MA packaging. The packaging materials used in experiment one were also used in this experiment. Additionally, every sample was contained in a CPET tray (described in 3.1.3), with the vacuum pouches surrounding the whole tray and sealed together. The duration of storage was also 20 days, with a storage temperature of 4 °C. Every analysis was performed on sampling day 0, 10 and 20, but microbial analysis and pH measurements were additionally performed on sampling day 5 and 15.

SGS-treatment

The SGS-treatment was carried out the same way according to experiment one (described in 3.1.1).

MA packaging

MA packaging was carried out using a chamber machine (described in 3.1.1) with the gas mixture set to 60 % CO₂ and 40 % N₂. The gas mixture was provided by the same gas mixer (described in 3.1.1) as for SGS-treatment. Samples (50±5g) were placed singularly into separate CPET trays (described in 3.1.3), placed into their respective vacuum pouches of the assigned material and filled with the MA and sealed. The SGS-treated samples were re-packed into MA packaging the day after, 18 hours after from the initial experiment start. The *g/p* ratio is assumed to be 4:1±5 %.

The three groups in experiment two without SGS-treatment will later be referred to as:

- **PA/PE MA** – samples stored in a MA with PA/PE material
- **BioLB MA** – samples stored in a MA with biobased material (low barrier)
- **BioHB MA** – samples stored in a MA with biobased material (high barrier)

The three groups in experiment two without SGS-treatment will later be referred to as:

- **PA/PE SGS+MA** – samples stored in a MA with PA/PE material
- **BioLB SGS+MA** – samples stored in a MA with biobased material (low barrier)
- **BioHB SGS+MA** – samples stored in a MA with biobased material (high barrier)

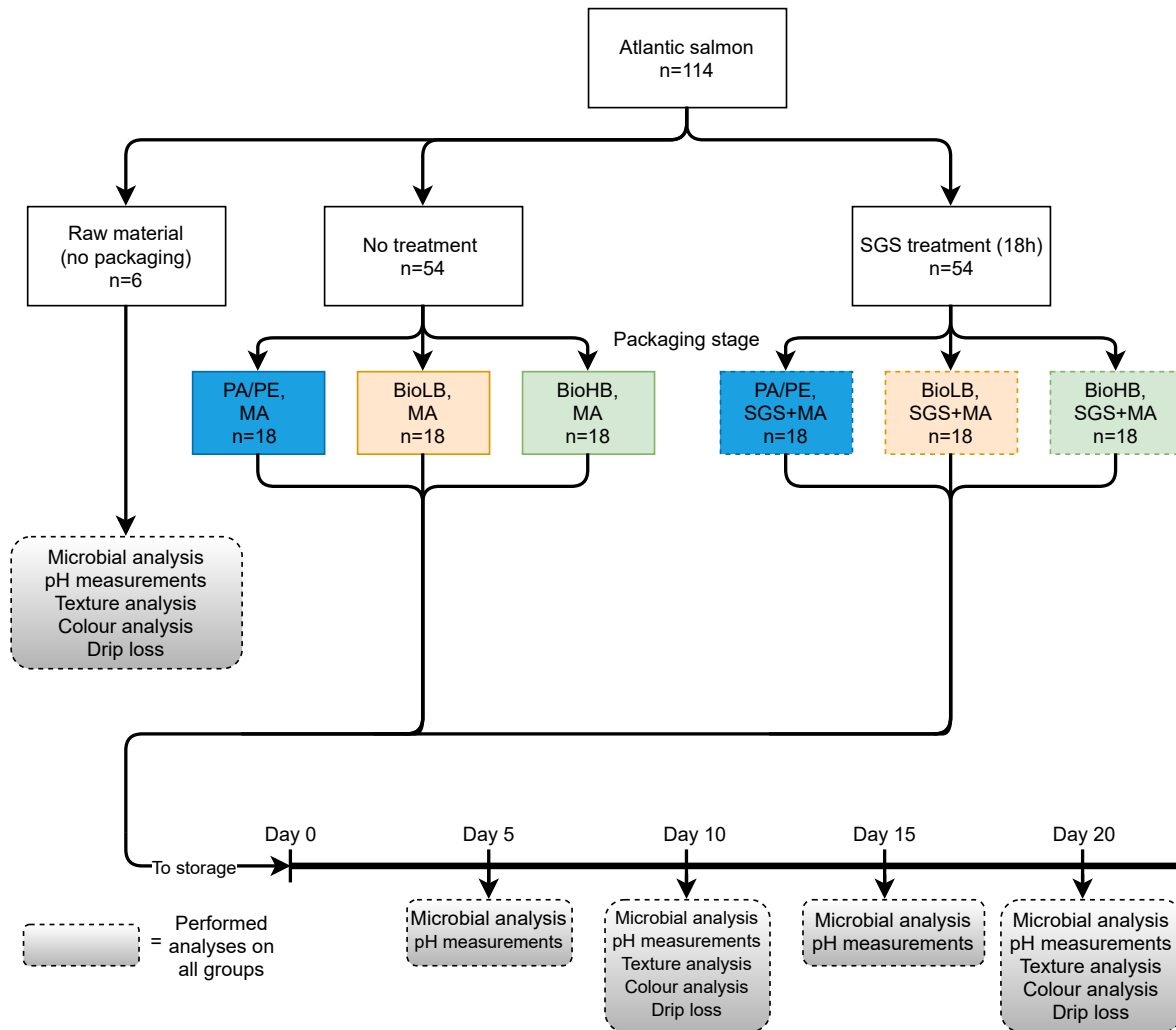


Figure 8: Illustration of the MA packaging experimental design. Raw material ($n=6$) was analysed day 0, all six groups ($n=3$ from each group each sampling day) at day 5, 10, 15 and 20 for microbial analysis, pH measurements. At sampling day 10 and 20 all six groups ($n=3$) were additionally analysed for texture and colour. The SGS-treated group were re-packed after 18 hours, and has one day delay.

3.1.3 Packaging Materials Used in Experiment One and Two

Biodegradable and Biobased Vacuum Pouches (low and high barrier)

Both biobased vacuum pouches were provided by Grounded Packaging (Sydney, Australia). The company has several certifications, such as FSC, ABA Home compost & Industrial, OK Home compost & Compost Industrial and BPI (Grounded Packaging, n.d.).

The low barrier vacuum pouch is a transparent single-layer film made from cassava root and corn derivatives blended with a copolymer (PBAT). The material is only partly biobased since PBAT is a petroleum-based resource, yet it is still biodegradable (European bioplastics, 2018). According to the technical datasheet (Appendix C.1), WVTR is 0 g/m²/24hrs (test method: ASTM E96), and OTR is 0 cc/m²/24hrs. The sealing temperature is ranged between 80-120 °C (1 sec). The dimension of the vacuum pouch is 250x200 mm (with a thickness of the film being 78-82 µm) and can be seen in Figure 9, along with the high barrier vacuum pouch.

The high barrier vacuum pouch is a transparent duplex film made of cellulose film laminated to BioPBS. The material is entirely biodegradable (European bioplastics, 2018). According to its technical datasheet (Appendix C.2), the WVTR is <14 g/m²/24hrs (test method: ASTM E96) and OTR is <1 g/m²/24hrs. The sealing temperature range is 80-140 °C (0.5 sec). The dimension of the vacuum pouch is 250x160 mm (with a thickness of the film being 44.1-53.9 µm). It is unknown if the BioPBS is 100 % made of biobased resources, as it can range from 54-100 %. The correct amount of biobased resources used is not expressed in the technical datasheet.



Figure 9: From left: BioHB vacuum pouch and BioLB vacuum pouch used in both experiments.

PA/PE

The petroleum-based vacuum pouches (Lietpak, Vilnius, Lithuania) is a transparent coextrusion of PA/PE film. It is non-biodegradable. The typical values for WVTR is $2.3 \text{ g/m}^2/24\text{hrs}$ (test method: ASTM F1770) and OTR is $\sim 52 \text{ cc/m}^2/24\text{hrs}$ (test method: ASTM D3985) (Appendix C.3). The sealing temperature range is $140\text{-}160 \text{ }^\circ\text{C}$. Dimension of the vacuum pouch is $200 \times 300 \text{ mm}$ (with a thickness of the film being $80 \pm 5 \text{ } \mu\text{m}$) and can be seen in Figure 10.



Figure 10: Vacuum pouch (PE/PA) used in both experiments.

CPET Trays

The plastic trays (C2125-1A, Færch Plast, Holstebro, Denmark) were used in experiment two to contain the salmon during storage in MA packaging. The material is made of CPET (Figure 11), which includes properties from certified and approved rPET for use in food packaging. The tray dimensions are 125.3 mm in length, 99.1 mm in width and 32.5 mm in depth, and has a volume capacity of 230 mL (Appendix C.4).



Figure 11: CPET trays used in experiment 2.

3.2 Analytical Parameters

3.2.1 Microbiological Parameters

Long and Hammer (L&H) agar, supplemented with 0.025 % (w/v) Fe(III)NH₄Citrat solution, was used to quantify the total viable count (TVC) following the NMKL method No. 184. The length of the experiment was 20 days, and sampling days were day 0 (raw material), 5, 10, 15 and 20 (n=3 per groups per sampling day).

Approximately 10g of muscle tissue was cut from each sample using a sterile blade and transferred to a sterile stomacher pouch. The sampled piece was diluted 1:10 with sterile buffered peptone water, and the mixture was homogenised (Masticator, IUL, Spain) for 60s. The homogenate was further diluted and prepared to the appropriate concentration with sterile

peptone water. 0.1 ml from each dilution was inoculated on prepared L&H agar plates and spread evenly over the surface with a sterile stick. The plates were incubated for 5 days at 15 °C. Plates with up to 300 colonies were selected for reading.

3.2.2 Physiochemical Parameters

pH measurements

Samples were measured with a pH meter (Testo206 pH2, Germany) in triplicate for each group. The pH meter was calibrated to buffers of pH 4.0 and 7.0, and analyses were recorded at the same samples used for microbial analysis. The mean value of each group samples (n=3) from each sampling day were calculated.

Drip loss

Six samples from each group were numbered and weighed on day 0. Triplicate measurements from each group were weighed again on day 10, and the last three on day 20. The salmon samples were taken out of their packaging and the excess fluid was gently wiped off with a paper towel. Drip loss (%) was calculated by using the following equation:

$$\text{Drip loss}(\%) = \frac{m_0 - m_t}{m_0} \times 100 \quad (\text{Equation 5})$$

Where m_0 is the initial weight (g) of the raw material and m_t is the weight (g) of the sample at day 10 or 20.

Surface colour

DigiEye® full system (VeriVide Ltd., UK) was used for colorimetric analysis. The system was connected to a DSLR camera (Nikon D80, 35 mm lens, Nikon Corp., Japan), and the images were analysed by DigiPix software (version 2.8, VeriVide Ltd., UK). The samples were placed in a lightbox (daylight, 6400K) and measured by the L*a*b* values. Colour analysis was performed on sampling days 0, 10 and 20, and texture analysis was performed on the same samples afterwards (n=3 per group per sampling day). The mean value of each group samples from each sampling day were calculated.

Texture

Texture Analyzer TA-XT® plus (Stable Micro Systems Ltd., UK) was used to perform texture analysis at sampling days 0, 10 and 20 (n=3 per groups per sampling day). Each sample was measured in two places by a puncture test transverse to the muscle fibre orientation of the salmon with a 12.7 mm flat-ended cylindrical probe. The resistance force (N) was recorded with a 5 kg load cell at a speed of 2 mm/s and was presented by the Texture Exponent Lite software (Stable Micro Systems Ltd., UK). The surface breaking force (N) and the force needed to press the cylindrical probe down to 60 % of the fillet thickness (N) was also recorded. The latter was used to describe the firmness of the fillet. Averaged measurements were used for the data analysis.

3.2.3 Biochemical Parameters

Degradation of ATP

HPLC analysis of degradation products of ATP were only performed in experiment one of VP samples. Due to limited time, and an instrument error, degradation of ATP was not measured on samples from experiment two.

To prepare supernatants for HPLC analysis, frozen samples (-80 °C) from each sampling day (n=3) was grated, and approximately 1.5g was transferred into centrifuge tubes. Each sample had two parallels (n=2, a total of n=220). 5.0 ml of trichloroacetic acid (7 %, C₂HCl₃O₂, VWR International) was then added into the centrifuge tubes before the samples were homogenised (12,000 rpm, 60 sec) by ULTRA-TURRAX® (T25 Digital, IKA®-Werke, Germany). The homogenates were centrifuged at 4800 rpm (15 min, 4 °C) using a ROTINA 420 R centrifuge (Hettich, Germany). The supernatants were collected in 15ml sterile tubes for enzymatic analysis and frozen down to -80 °C until further use.

The applied analytical HPLC system was an Agilent 1260 Infinity II attached to a 1260 Infinity II Diode Array Detector HS (Agilent Technologies) with a Poroshell 120 porous column (EC-C18 3.0 x100mm, porous size 2.7µm, with a Poroshell 120 Fast Guard (3.0 x 5mm, Sub-2 µm), Agilent InfinityLab). The column had a temperature of 20 °C. Monopotassium phosphate (KH₂PO₄, 0.215 M) and Tetrabutylammonium hydrogen sulphate ([CH₃(CH₂)₃]₄N(HSO₄), 0.0023 M) mixed with 3.5 % liquid Acetonitrile (pH adjusted to 6.25 using 1.0 M potassium hydroxide (KOH)) was used as the solvent to the mobile phase. The flow was set at 0.2 ml/min

between 0-2 minutes, 0.8 ml/min between 2-9 minutes, and back to 0.2 ml/min between the last 9-10 minutes.

The frozen supernatants (-80 °C) were thawed and further transferred into 1.5 ml standard glass vials for HPLC (32 x 11.6 mm, VWR International) through filtration using a 25mm Syringe Filter with 0.2 µm Polyethersulfone membrane (VWR International). The samples were then analysed with the HPLC system to quantify ATP's degradation products. They were detected at 210 nm (ATP and ADP) and 260 nm (AMP, IMP, HxR and Hx). Commercial standards were used for quantification and to detect the retention time of the degradation products. The commercial standards are as follows: ATP (Sigma-Aldrich, CAS No.: 51963-61-2), ADP (Sigma-Aldrich, CAS No.: 20398-34-9), AMP (Sigma-Aldrich, CAS No.: 149022-20-8), IMP (Sigma-Aldrich, CAS No.: 352195-40-5), HxR (Sigma-Aldrich, CAS No.: 58-63-9), and Hx (Sigma-Aldrich, CAS No.: 68-94-0). The results were expressed as µmol/g sample and calculated in Excel.

3.2.4 Headspace Gas Analysis (CO₂ and O₂)

The composition of CO₂, O₂ and the remaining balance (N₂) in the packaging headspace gas was measured using an oxygen/carbon dioximeter (PBI Dansensor, CheckMate 9900, Ringsted, Denmark). A sample volume of 2ml was assembled from the headspace by injecting a syringe through the packaging film. To avoid the plastic from rupturing while injecting, a rubber septum (Nordic Supply, Skodje, Norway) was placed on the film where probing was performed. Measurements were done in triplicate for each packaging material on each sampling day. On day 0, just before the salmon were packed, the headspace composition was measured in six empty trays to establish an accurate starting point.

3.3 Statistics

Data from both experiments were analysed in IBM® SPSS® Statistics Version 26 (IBM, New York, USA). Statistical analyses were performed with univariate analysis using the general linear model (GLM). GLM procedure was used to conduct a full-factorial analysis. One-way ANOVA (Tukey HSD post-hoc multiple comparison assumed) was also performed to calculate the significance level between the groups. This was also used to calculate any differences between groups with SGS-treatment and traditional VP or MA packaging only, and to compare the packaging materials regardless of SGS-treatment. All measured variables were analysed as

dependent variables, with storage days as a split factor and with experimental group as a fixed factor to test the subject's effect over time. The alpha level was set to 5 % ($p < 0.05$), and the results are presented as the mean value of three parallels with a standard deviation (\pm) for each group per sampling day. The results are given in text and tables or presented in plots.

4. Results

This study included two experiments that were performed in two runs based on packaging method. The results from each experiment are presented separately. The main essence is to highlight how the traditional VP or MA packed groups with biobased and biodegradable materials performed in regard to their control group with PA/PE material. Additionally, the results of SGS-treatment in each experiment are presented in regard to its SGS-treated control group with PA/PE material.

4.1 Experiment One – Vacuum Packaging Combined with Soluble Gas Stabilisation

4.1.1 Microbial Growth

The microbial growth (TVC) was found affected by the experimental groups (GLM, $p < 0.001$, Figure 12), showing increased microbial growth as a function of storage time (GLM, $p < 0.001$) and differences in TVC between the experimental groups (GLM, $p < 0.001$). Moreover, a significant interaction between the groups and storage time was observed (GLM, $p < 0.001$). The main difference of TVC between groups was observed at day five, showing no bacterial growth on salmon packaged in BioHB SGS+vacuum (Figure 12). Furthermore, all SGS-treated groups have, on average, a significant lower TVC at day five compared to those packaged in traditional VP (GLM, $p < 0.004$). In contrast, BioLB SGS+vacuum had a significantly higher TVC at day 20 compared to the other SGS-treated groups (GLM, $p < 0.001$), which resulted in a significant difference (GLM, $p < 0.048$) between the packaging materials (regardless of SGS) at day 20 (Appendix A.1). The BioHB material was found to be comparable with the PA/PE material throughout storage within both traditional VP and SGS-treated groups. The BioLB material was additionally found to be similar to the PA/PE material for traditional VP.

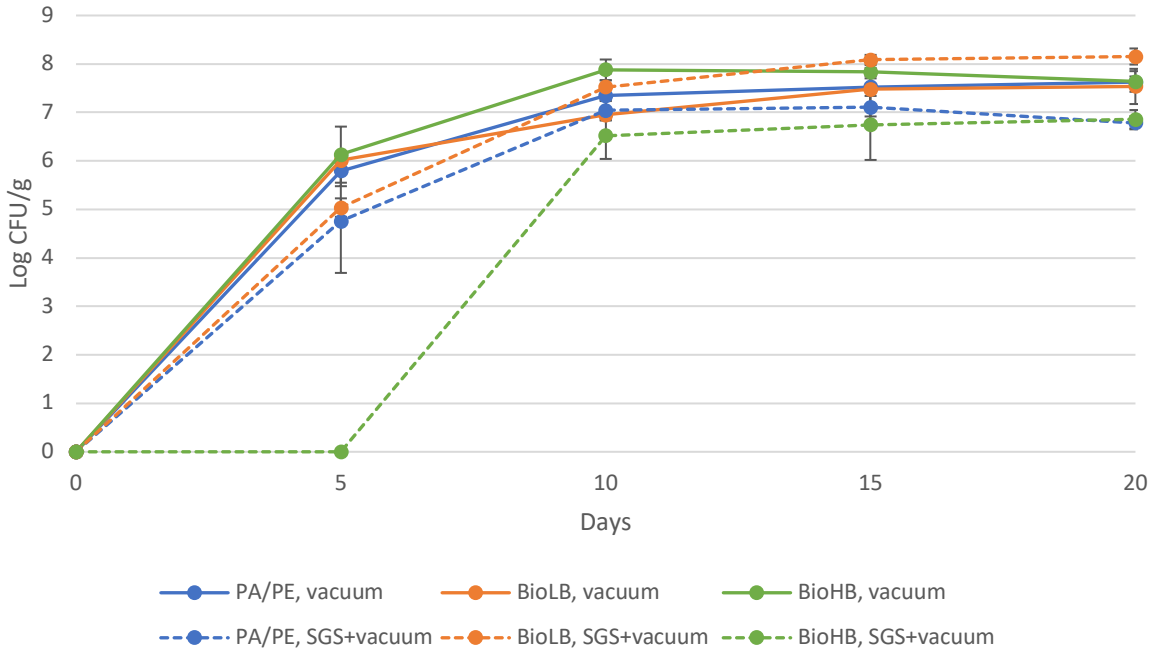


Figure 12: Main effects of packaging material (SGS and vacuum only, $n=108$) on TVC (log CFU/g \pm SE) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment one). GLM: $p_{\text{groups}} < 0.001$, $p_{\text{storage}} < 0.001$, $p_{\text{interaction}} < 0.001$.

4.1.2 pH Measurements

The pH levels were found significantly affected by the experimental groups (GLM, $p < 0.001$), whereas the groups had significant differences amongst them throughout the experiment (GLM, $p < 0.001$, Table 1). The pH level as a function of storage time was not affected, nor was an interaction between experimental groups and storage detected (GLM, $p = 0.409$, $p = 0.310$, respectively). SGS-treated groups had, on average, a higher pH level on day 10 compared to traditional VP (GLM, $p < 0.014$), but this was found insignificant for the remaining storage days. The two control groups with PA/PE material were not significantly different from either BioLB or BioHB, but the two biobased materials gave significant differences amongst them for the three sampling days (GLM, $p < 0.044$). BioHB vacuum continued to have a lower pH level than the other experimental groups, while BioLB SGS+vacuum had the highest pH level amongst the rest (Table 1, Appendix A.2).

Table 1: Main effects of packaging material (SGS and vacuum only, n=60) on pH values (\pm SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment one). GLM: $p_{groups}<0.001$, $p_{storage}=0.409$, $p_{interaction}=0.310$. The different superscripts (^{ab}) indicate the significant variations ($p<0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

Day	Groups						p-value ¹
	PA/PE vacuum	BioLB vacuum	BioHB vacuum	PA/PE SGS+ vacuum	BioLB SGS+ vacuum	BioHB SGS+ vacuum	
1	6.14 \pm 0.01	6.14 \pm 0.01	6.14 \pm 0.01	6.14 \pm 0.01	6.14 \pm 0.01	6.14 \pm 0.01	
10	6.16 \pm 0.05 ^a	6.17 \pm 0.01 ^a	6.11 \pm 0.06 ^a	6.22 \pm 0.06 ^{ab}	6.33 \pm 0.01 ^b	6.16 \pm 0.03 ^a	<0.001
15	6.22 \pm 0.11 ^{ab}	6.21 \pm 0.04 ^{ab}	6.02 \pm 0.09 ^a	6.16 \pm 0.06 ^{ab}	6.27 \pm 0.11 ^b	6.10 \pm 0.03 ^{ab}	0.025
20	6.13 \pm 0.06	6.23 \pm 0.09	6.07 \pm 0.05	6.22 \pm 0.06	6.25 \pm 0.10	6.17 \pm 0.02	0.063

¹Significance level $p<0.05$, GLM Univariate

Significance between groups with and without SGS-treatment (pH)		Significance between packaging materials regardless of SGS-treatment (pH)				
Day	p-value ¹	Day	Packaging material			p-value ¹
10	0.014	10	PA/PE ^{ab}	BioLB ^b	BioHB ^a	0.033
15	0.614	15	PA/PE ^b	BioLB ^b	BioHB ^a	0.004
20	0.097	20	PA/PE ^{ab}	BioLB ^b	BioHB ^a	0.044

4.1.3 Drip Loss

The drip loss (%) was found affected by the experimental design (GLM, $p<0.001$, Figure 13), showing increased drip loss as a function of storage time (GLM, $p<0.001$) and differences in drip loss between the experimental groups were also found (GLM, $p<0.001$, Appendix A.3). Along with a significant interaction between the groups and storage time (GLM, $p<0.010$), the SGS-treatment also resulted in a higher drip loss than traditional VP on sampling day 10 and 20 (GLM, $p<0.002$, Appendix A.3). Out of the SGS-treated groups, BioHB SGS+vacuum resulted in the lowest drip loss (5.48 \pm 0.53 %) on day 20, while the SGS control group with PA/PE material had the highest drip loss (7.68 \pm 0.20 %), closely followed by BioLB SGS+vacuum (7.64 \pm 0.69 %). The experimental groups with traditional VP were insignificant from each other throughout storage time. The packaging materials, regardless of SGS, gave comparable results throughout storage (GLM, $p>0.051$, Appendix A.3).

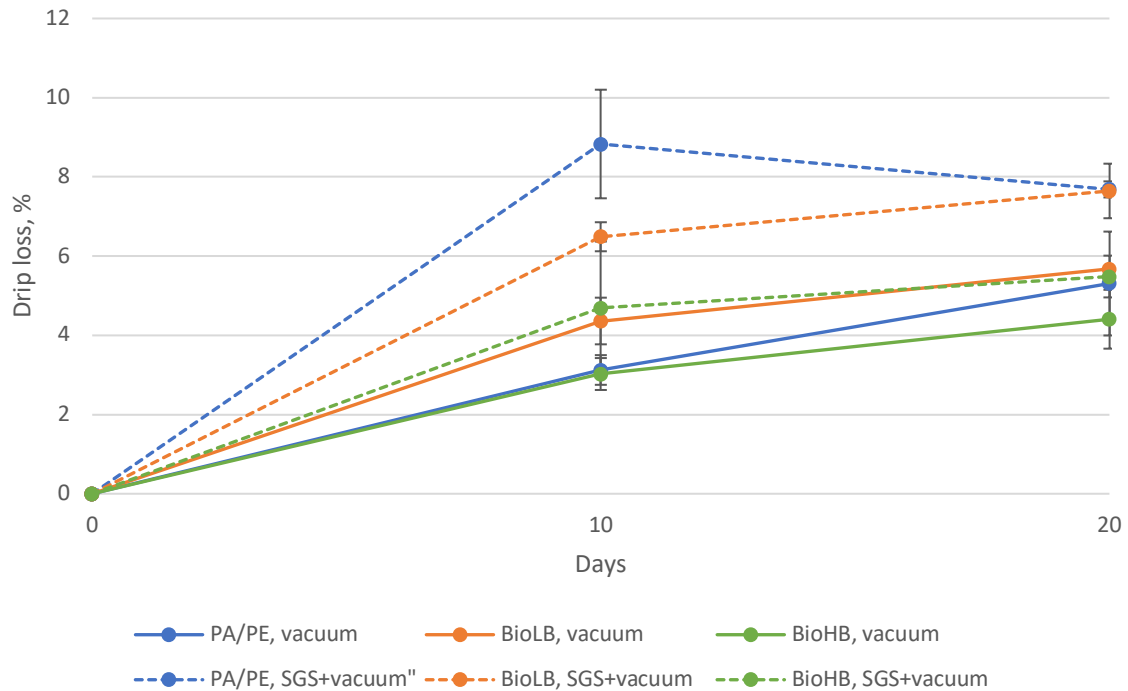


Figure 13: Main effects of packaging material (SGS and vacuum only, $n=36$) on drip loss ($\% \pm SD$) during 20 days of storage ($4\text{ }^{\circ}\text{C}$) of fresh Atlantic salmon (experiment one). GLM: $p_{\text{groups}} < 0.001$, $p_{\text{storage}} < 0.001$, $p_{\text{interaction}} < 0.010$.

4.1.4 Colour Measurements

The lightness (L) was found to be affected by the experimental groups (GLM, $p < 0.002$, Table 2), showing developments of lightness on the surface of the fish muscle between the groups. The development was not affected as a function of storage time (GLM, $p = 0.667$), but there was observed an interaction between groups and storage time (GLM, $p < 0.046$). Every experimental group had a slightly increased lightness throughout the experiment, whereas samples with BioHB material was significantly lighter (GLM, $p < 0.017$) than samples from PA/PE material on day 10. Meanwhile, samples from BioLB were between the other materials, but the difference between these materials was found to be insignificant (GLM, $p = 0.435$) at the end of storage. The SGS-treatment alone did not affect the lightness in any significant way (GLM, $p = 0.069$, $p = 0.074$, Table 2).

The raw material from experiment one started with a redness (a^*) at 17.34 ± 1.33 (Table 2) and was affected as a function of storage time (GLM, $p < 0.001$), where the redness increased towards sampling day 10 but had a slight decrease towards the end of storage. Samples with BioLB material were redder than samples with PA/PE, whereas BioHB was in between, yet insignificant (GLM, $p = 0.051$). The packaging materials were comparable at day 20, and no

differences between the groups were observed (GLM, $p=0.557$, $p=0.435$, respectively). SGS-treatment did not affect the redness any more than traditional VP (GLM, $p>0.377$), but BioLB SGS+vacuum was significantly more red than PA/PE SGS+vacuum on day 10.

The yellowness (b^*) was found affected by the experimental groups (GLM, $p<0.001$, Table 2), showing increasing yellowness as a function of storage time (GLM, $p<0.005$), and additional differences in yellowness between the experimental groups (GLM, $p<0.004$). Moreover, there was observed no interaction between groups and storage time (GLM, $p=0.084$). Samples with BioHB material were significantly more yellow than samples with PA/PE material on day 10 (GLM, $p<0.008$), but the differences were found to be insignificant at the end of storage. SGS-treatment did not give more or less yellowness to the samples than traditional VP on either day (GLM, $p>0.370$). PA/PE SGS+vacuum was significantly less yellow than the biobased SGS-treated groups throughout storage (GLM, $p<0.004$).

Table 2: Main effects of packaging material (SGS and vacuum only, $n=46$) on colorimetric values ($L/a^*/b^*\pm SD$) during 20 days of storage (4°C) of fresh Atlantic salmon (experiment one). GLM: L ($p_{\text{groups}}<0.002$, $p_{\text{storage}}=0.667$, $p_{\text{interaction}}<0.046$), a^* ($p_{\text{groups}}=0.983$, $p_{\text{storage}}<0.001$, $p_{\text{interaction}}=0.182$), b^* ($p_{\text{groups}}<0.001$, $p_{\text{storage}}<0.005$, $p_{\text{interaction}}<0.084$). The different superscripts (abc) indicates the significant variation ($p<0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

Day	Groups						p-value ¹
	PA/PE vacuum	BioLB vacuum	BioHB vacuum	PA/PE SGS+ vacuum	BioLB SGS+ vacuum	BioHB SGS+ vacuum	
L	Initial*	58.46±0.45	58.46±0.45	58.46±0.45	58.46±0.45	58.46±0.45	
	10	61.55±0.23 ^{abc}	61.28±0.68 ^{ab}	62.47±0.56 ^c	60.45±0.41 ^a	61.38±0.12 ^{abc}	0.002
	20	61.47±0.95	61.83±0.28	62.58±0.07	61.80±0.44	60.45±0.96	0.127
a*	Initial*	17.34±1.33	17.34±1.33	17.34±1.33	17.34±1.33	17.34±1.33	
	10	21.71±0.21 ^{abc}	21.99±0.10 ^{abc}	22.39±0.50 ^{bc}	21.35±0.44 ^a	22.52±0.28 ^c	0.009
	20	19.12±1.72	19.45±0.17	19.14±0.52	20.07±0.24	18.70±0.09	0.435
b*	Initial*	7.59±1.83	7.59±1.83	7.59±1.83	7.59±1.83	7.59±1.83	
	10	10.09±0.31 ^b	9.98±0.66 ^b	10.27±0.61 ^b	6.88±0.57 ^a	9.75±0.25 ^b	<0.001
	20	11.88±1.59 ^{bc}	10.78±0.36 ^{abc}	10.09±1.11 ^{ab}	9.01±0.07 ^a	12.59±0.61 ^c	0.004

*Day 0, raw material before packaging

¹Significance level $p<0.05$, GLM Multivariate

Significance between groups with and without SGS-treatment (L, a*, b*)			Significance between packaging materials regardless of SGS-treatment (colour)				
Day		p-value ¹	Day	Parameter	Packaging material		p-value ¹
	L	0.069		L	PA/PE ^a	BioLB ^{ab}	0.017
10	a*	0.377	10	a*	PA/PE ^a	BioLB ^b	0.051
	b*	0.370		b*	PA/PE ^a	BioHB ^b	0.008
	L	0.074		L	PA/PE	BioLB	0.435
20	a*	0.579	20	a*	PA/PE	BioHB	0.557
	b*	0.987		b*	PA/PE	BioLB	0.261

4.1.5 Texture

The resistance strength (N) was not significantly affected by the experimental groups (GLM, $p=0.362$, Table 3), but showed a decreasing resistance force as a function of storage time (GLM, $p<0.031$). The different packaging materials did not affect the resistance force in any significant way (GLM, $p>0.345$), which indicate that they developed comparable results amongst them. SGS-treatment did not give any further effect than traditional VP (GLM, $p>0.575$), whereas the SGS-treated biobased groups also had similar results compared to the SGS-treated control group with PA/PE material.

Breaking force (N) was found affected by the experimental groups (GLM, $p<0.004$, Table 3), showing a general reduction of force needed to break the surface as a function of storage time (GLM, $p<0.015$), except for BioLB SGS+vacuum which had a slight increase from initial start. There was observed no difference between the groups (GLM, $p>0.053$), and no interaction between groups and storage time (GLM, $p=0.643$). The packaging materials did not have any significant differences amongst them (GLM, $p>0.126$), and SGS-treatment did not differ from traditional VP either (GLM, $p>0.277$).

The firmness (N) was also found affected by the experimental groups (GLM, $p<0.002$, Table 3), with a decreasing firmness as function of storage time (GLM, $p<0.001$). The same exception was observed for this textural property, whereas BioLB SGS+vacuum was the only group that resulted with an increased firmness from initial start. There was also observed a significant difference amongst the experimental groups on day 10 (GLM, $p<0.041$) where BioHB SGS+vacuum had the least firmness, while control group PA/PE SGS+vacuum had the highest firmness value (Table 3). This resulted in a significant difference between the packaging materials on day 10 (GLM, $p<0.036$), followed by insignificance between SGS-treated groups and traditional VP (GLM, $p=0.430$).

Table 3: Main effects of packaging material (SGS and vacuum only, n=42) on textural parameters (RF/BF/F (N) ± SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment one). GLM: RF ($p_{groups}=0.362$, $p_{storage}<0.031$, $p_{interaction}<0.931$), BF ($p_{groups}<0.015$, $p_{storage}<0.004$, $p_{interaction}=0.643$), F ($p_{groups}<0.002$, $p_{storage}<0.001$, $p_{interaction}<0.661$). The different superscripts (^{ab}) indicates the significant variation ($p<0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

	Day	Groups						p-value ¹
		PA/PE vacuum	BioLB vacuum	BioHB vacuum	PA/PE SGS+ vacuum	BioLB SGS+ vacuum	BioHB SGS+ vacuum	
Resistance force (N)	Initial*	6.428±1.66	6.428±1.66	6.428±1.66	6.428±1.66	6.428±1.66	6.428±1.66	
	10	6.408±1.73	5.888±1.02	5.897±0.38	6.237±1.33	6.073±0.27	5.087±0.54	0.700
	20	5.112±0.88	5.082±0.62	5.466±0.79	4.991±0.77	5.901±0.89	4.205±0.18	0.191
Breaking force (N)	Initial*	7.450±1.53	7.450±1.53	7.450±1.53	7.450±1.53	7.450±1.53	7.450±1.53	
	10	7.100±0.96 ^{ab}	7.155±0.69 ^{ab}	7.198±0.11 ^{ab}	8.237±1.03 ^b	7.483±0.78 ^{ab}	5.996±0.67 ^a	0.075
	20	5.770±1.06 ^{ab}	5.846±0.22 ^{ab}	5.967±0.92 ^{ab}	6.573±0.96 ^{ab}	7.713±1.36 ^b	5.079±0.07 ^a	0.053
Firmness (N)	Initial*	6.26±0.98	6.26±0.98	6.26±0.98	6.26±0.98	6.26±0.98	6.26±0.98	
	10	6.94±0.87 ^{ab}	6.60±0.40 ^{ab}	6.49±0.15 ^{ab}	7.88±0.66 ^b	7.48±1.07 ^{ab}	5.75±0.76 ^a	0.041
	20	5.11±1.34	5.06±0.11	4.55±1.39	6.17±1.01	7.16±1.50	4.72±0.21	0.084

*Day 0, raw material before packaging

¹Significance level $p<0.05$, GLM Multivariate

Significance between groups with and without SGS-treatment (Resistance force, Breaking force, Firmness)			Significance between packaging materials regardless of SGS-treatment (texture)					
Day		p-value ¹	Day	Parameter	Packaging material			p-value ¹
10	RF (N)	0.575	10	Resistance Force (N)	PA/PE	BioLB	BioHB	0.345
	BF (N)	0.850		Breaking Force (N)	PA/PE	BioLB	BioHB	0.126
	Firmness (N)	0.430		Firmness (N)	PA/PE ^b	BioLB ^{ab}	BioHB ^a	0.036
20	RF (N)	0.638	20	Resistance Force (N)	PA/PE	BioLB	BioHB	0.382
	BF (N)	0.277		Breaking Force (N)	PA/PE	BioLB	BioHB	0.157
	Firmness (N)	0.072		Firmness (N)	PA/PE	BioLB	BioHB	0.137

4.1.6 H-value

The H-values (%), calculated from the HPLC analysis, were found to be affected by the experimental groups (GLM, $p < 0.001$, Table 4), showing increased H-values as a function of storage time (GLM, $p < 0.001$). The interaction between groups and storage time was also detected as significant (GLM, $p < 0.029$). BioLB SGS+vacuum had a significantly higher H-value than control group PA/PE SGS+vacuum on day 20 (GLM, $p < 0.001$), while BioHB SGS+vacuum had a slightly (insignificantly) lower H-value than the control. Control group for traditional VP, PA/PE vacuum, was not significantly different from either groups during storage. Furthermore, the biobased materials did not differ from PA/PE during storage time (GLM, $p > 0.379$), neither were there differences between SGS-treatment and traditional VP (GLM, $p > 0.150$).

Table 4: Main effects of packaging material (SGS and vacuum only, $n=180$) on H-values ($\pm SD$) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment one). GLM: $p_{groups} < 0.001$, $p_{storage} < 0.001$, $p_{interaction} < 0.029$. The different superscripts (^{ab}) indicates the significant variation ($p < 0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

Day	Groups						p-value ¹
	PA/PE, vacuum	BioLB, vacuum	BioHB, vacuum	PA/PE, SGS+ vacuum	BioLB, SGS+ vacuum	BioHB, SGS+ vacuum	
Initial*	0	0	0	0	0	0	
5	13.7±0.24	13.4±2.88	18.1±5.32	17.9±5.55	20.8±7.65	16.5±3.07	0.436
10	31.8±2.87	22.6±7.12	44.1±17.13	29.1±5.17	32.6±2.46	26.9±1.84	0.093
15	47.0±18.10 ^{ab}	39.1±1.79 ^a	69.8±7.80 ^b	41.4±5.56 ^a	56.5±4.18 ^{ab}	40.3±9.31 ^a	0.011
20	62.1±4.48 ^{ab}	49.0±17.89 ^a	80.1±5.33 ^b	48.5±2.40 ^a	75.7±9.74 ^b	44.6±3.67 ^a	<0.001

*Day 0, raw material

¹Significance level $p < 0.05$, GLM Univariate

Significance between groups with and without SGS-treatment (H-value)		Significance between packaging materials regardless of SGS-treatment (H-value)				
Day	p-value ¹	Day	Packaging material		p-value ¹	
5	0.150	5	PA/PE	BioLB	BioHB	0.867
10	0.486	10	PA/PE	BioLB	BioHB	0.379
15	0.381	15	PA/PE	BioLB	BioHB	0.404
20	0.340	20	PA/PE	BioLB	BioHB	0.706

4.2 Experiment Two – Modified Atmosphere Packaging Combined with Soluble Gas Stabilisation

4.2.1 Headspace Gas (CO₂ and O₂)

The CO₂ (%) level was affected by the experimental groups (GLM, $p < 0.001$, Figure 14A), showing predominantly decreasing CO₂ levels for experimental groups with BioLB as a function of storage time (GLM, $p < 0.001$). There was also observed an interaction between groups and storage time (GLM, $p < 0.001$), along with significant differences between the groups at every sampling day (GLM, $p < 0.001$, Appendix B.4). Both BioLB groups decreased rapidly throughout storage time, while the BioHB material was insignificant compared to the control group of PA/PE (GLM, $p < 0.001$). There was no difference between SGS-treated groups compared to traditional MA groups (GLM, $p > 0.929$), as a result of both BioLB groups' poor performance. BioHB SGS+MA had a significantly higher CO₂ level (56.1 ± 0.4 %) than control group PA/PE SGS+MA (54.3 ± 0.17 %) at sampling day 20, whereas the initial CO₂ composition was at 58.6 ± 0.7 % (Appendix B.4).

The O₂ (%) level was also affected by the experimental groups (GLM, $p < 0.001$, Figure 14B), whereas the O₂ level for BioLB groups for the most part increased as a function of storage time (GLM, $p < 0.001$). PA/PE MA was observed to have 0.53 ± 0.83 % O₂ level at day five, where the standard deviation indicates that only one of three parallels contained a higher level than the rest (Figure 13B, Appendix B4). There was detected an interaction between groups and storage time as well (GLM, $p < 0.001$), showing BioLB groups to continuously permeate O₂ into the package during storage. Consequently, BioLB material was significantly different from both control groups with PA/PE material and BioHB material at every sampling day (GLM, $p < 0.007$), except for day 20 (GLM, $p = 0.118$). Experimental groups with BioHB material exceeded above control group PA/PE, with an average intake of O₂ into the package of 0.02 %, compared to PA/PE groups which had an average of 0.17 %. The effect of SGS-treatment was not significant (GLM, $p > 0.152$, Appendix B.4), due to the significance between the packaging materials instead.

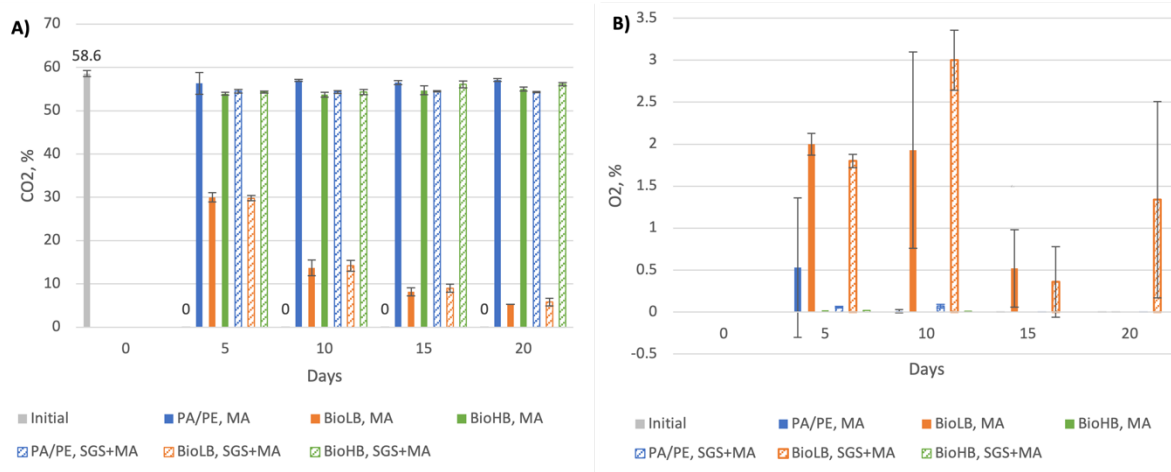


Figure 14: Main effects of packaging material (SGS and MA only, $n=90$) on CO₂ (A) and O₂ (B) (%±SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). **A:** GLM: CO₂ ($p_{groups}<0.001$, $p_{storage}<0.001$, $p_{interaction}<0.001$). **B:** GLM: O₂ ($p_{groups}<0.001$, $p_{storage}<0.001$, $p_{interaction}<0.001$).

4.2.2 Microbial Growth

The microbial growth (TVC) was found to be affected by the experimental groups (GLM, $p<0.001$, Figure 15), showing increased microbial growth as a function of storage time (GLM, $p<0.001$), along with an interaction between groups and storage time (GLM, $p<0.001$). The experimental groups had a significant difference from each other from day 10 (GLM, $p<0.001$), showing both BioLB groups with a higher development of TVC compared to both control groups of PA/PE and BioHB groups (Figure 15, Appendix B.1). Experimental groups with BioHB material had significantly lower TVC than control groups with PA/PE on sampling day 15 and 20 (GLM, $p<0.001$). Because of the high microbial growth in both groups with BioLB, there was no significant difference observed between SGS-treatment and traditional MA packaging (GLM, $p>0.439$).

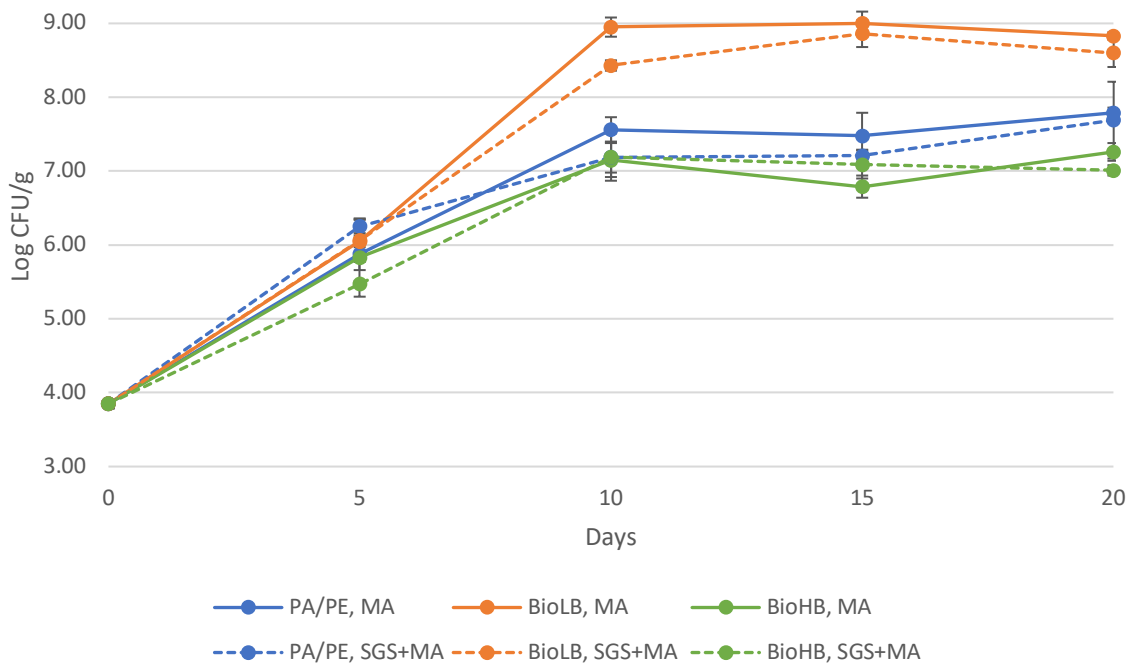


Figure 15: Main effects of packaging material (SGS and MA only, $n=108$) on TVC ($\log CFU/g \pm SE$) during 20 days of storage ($4^\circ C$) of fresh Atlantic salmon (experiment two). GLM: $p_{groups} < 0.001$, $p_{storage} < 0.001$, $p_{interaction} < 0.001$

4.2.3 pH Measurements

The pH level was affected by the experimental groups (GLM, $p < 0.001$, Table 5), showing different development amongst the groups from day 10 (GLM, $p < 0.001$) as a function of storage time (GLM, $p < 0.001$). Moreover, a significant interaction between groups and storage time was observed (GLM, $p < 0.001$). Experimental groups with BioLB material had a significantly higher pH level compared to control groups with PA/PE material and BioHB material from day 10 onwards (GLM, $p < 0.017$, Table 5). Experimental groups with BioHB material had a similar decrease in pH level as control groups with PA/PE material, and was found insignificant from each other (Table 5, Appendix B.2). As a consequence of both experimental groups with BioLB having a significantly higher pH level throughout storage time, there was found no significant difference between SGS-treatment and traditional MA packaging (GLM, $p > 0.054$).

Table 5: Main effects of packaging material (SGS and MA only, n=60) on pH values (\pm SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: $p_{\text{groups}} < 0.001$, $p_{\text{storage}} < 0.001$, $p_{\text{interaction}} < 0.001$. The different superscripts (^{abcd}) indicates the significant variation ($p < 0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

pH	Day	Groups						p-value ¹
		PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+MA	BioLB, SGS+MA	BioHB, SGS+MA	
	Initial*	6.31 \pm 0.04	6.31 \pm 0.04	6.31 \pm 0.04	6.31 \pm 0.04	6.31 \pm 0.04	6.31 \pm 0.04	
	5	6.16 \pm 0.07	6.17 \pm 0.03	6.10 \pm 0.05	6.09 \pm 0.02	6.14 \pm 0.03	6.11 \pm 0.02	0.114
	10	6.06 \pm 0.05 ^{ab}	6.20 \pm 0.06 ^{cd}	6.03 \pm 0.04 ^a	6.10 \pm 0.04 ^{abc}	6.28 \pm 0.03 ^d	6.16 \pm 0.00 ^{bc}	<0.001
	15	6.03 \pm 0.06 ^{ab}	6.18 \pm 0.06 ^c	6.12 \pm 0.02 ^{bc}	6.05 \pm 0.03 ^{ab}	6.12 \pm 0.05 ^{bc}	5.98 \pm 0.02 ^a	<0.001
	20	6.00 \pm 0.04 ^a	6.18 \pm 0.05 ^b	5.98 \pm 0.03 ^a	5.97 \pm 0.05 ^a	6.09 \pm 0.09 ^{ab}	6.00 \pm 0.01 ^a	<0.001

*Day 0, raw material before packaging (n=3)

¹Significance level $p < 0.05$, GLM Univariate

Significance between groups with and without SGS-treatment (pH)		Significance between packaging materials regardless of SGS-treatment (pH)				
Day	p-value ¹	Day	Packaging material			p-value ¹
5	0.206	5	PA/PE	BioLB	BioHB	0.141
10	0.054	10	PA/PE ^a	BioLB ^b	BioHB ^a	<0.001
15	0.089	15	PA/PE ^a	BioLB ^b	BioHB ^a	0.017
20	0.439	20	PA/PE ^a	BioLB ^b	BioHB ^a	<0.001

4.2.4 Drip Loss

Drip loss (%) was not affected by the experimental design (GLM, $p=0.050$, Figure 16), but showed an increasing drip loss as a function of storage time (GLM, $p < 0.020$). Consequently, there was no interaction between groups and storage time (GLM, $p=0.781$). There were no significant differences between the two biobased materials compared to control groups with PA/PE (GLM, $p=369$, Appendix B.3), nor was there differences between the groups (GLM, $p > 0.081$). This indicates that the experimental groups with biobased material had comparable drip loss to control groups with PA/PE material. SGS-treatment was found to have higher drip loss than traditional MA packaging on day 10 (GLM, $p < 0.006$), but was found to be insignificant on day 20 (GLM, $p=0.102$, Appendix B.3).

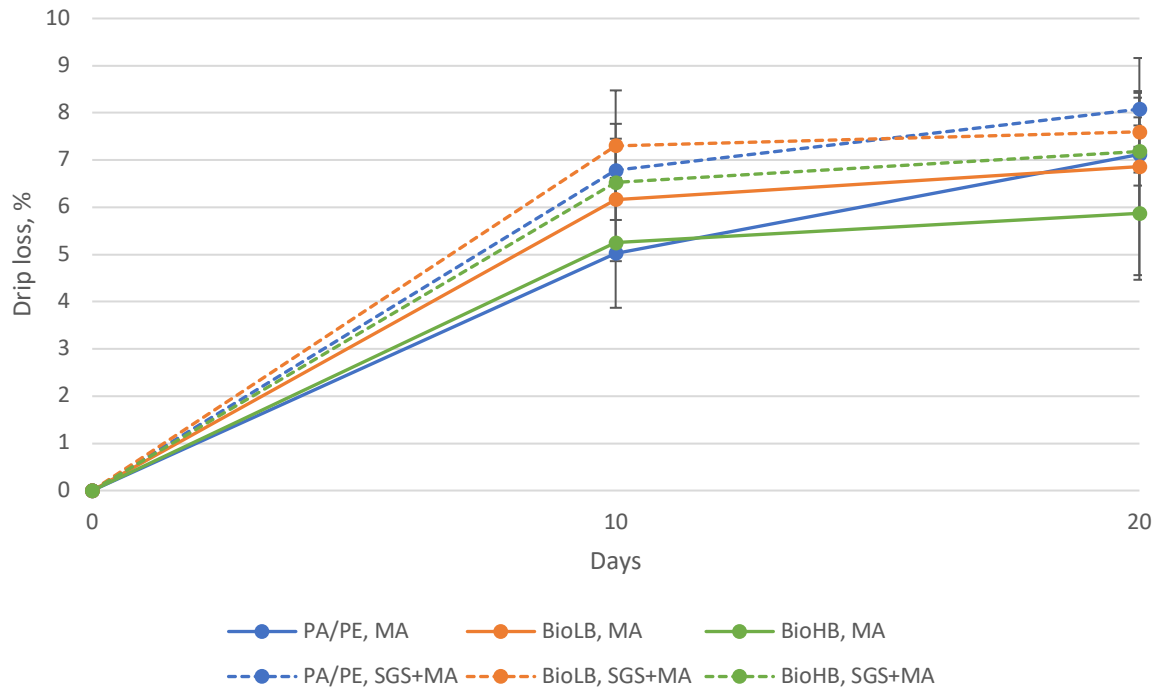


Figure 16: Main effects of packaging material (SGS and MA only, $n=36$) on drip loss ($\% \pm SD$) during 20 days of storage (4°C) of fresh Atlantic salmon (experiment two). GLM: $p_{\text{groups}}=0.050$, $p_{\text{storage}}<0.020$, $p_{\text{interaction}}<0.781$.

4.2.5 Colour Measurements

The lightness (L) was found affected by the experimental groups (GLM, $p<0.001$, Table 6), showing different developments of surface lightness as a function of storage time (GLM, $p<0.001$). In spite of this, there was observed no interaction between groups and storage time (GLM, $p=0.395$). The experimental groups with SGS-treatment had on average a lighter appearance than traditional MA packaging on day 10 (GLM, $p<0.026$), additionally showing differences between groups that day (GLM, $p<0.011$) whereas the SGS-treated groups had more lightness than traditional MA packaging. Experimental groups with biobased material was comparable to control groups with PA/PE material throughout storage time (GLM, $p>0.190$).

Redness (a^*) was affected by the experimental groups (GLM, $p<0.004$, Table 6), but showed no increased redness as a function of storage time (GLM, $p=0.147$), nor was there an interaction between groups and storage (GLM, $p=0.601$). PA/PE MA was significantly less red than every experimental group on day 10 (GLM, $p<0.001$), but there were no significant differences amongst the packaging materials on the remaining sampling days (GLM, $p>0.066$). SGS-treatment did not differ from traditional MA packaging (GLM, $p>0.211$), showing that the experimental groups with biobased material gave comparable results to the control groups with PA/PE material.

The yellowness (b*) was also found affected by the experimental groups (GLM, $p < 0.013$, Table 6), showing increasing yellowness as a function of storage time (GLM, $p < 0.002$). There was not observed any interaction between groups and storage time (GLM, $p = 0.250$). BioLB MA was significantly more yellow than control group PA/PE MA on day 10 (GLM, $p < 0.002$), but this was found insignificant at the end of storage. No differences between the packaging materials (GLM, $p > 0.229$) indicates that the biobased materials had similar development of yellowness compared to the control groups with PA/PE. Furthermore, SGS-treatment was on average more yellow than traditional MA packaging on day 10 (GLM, $p < 0.043$), but was found insignificant at the end of storage time (GLM, $p = 0.434$).

Table 6: Main effects of packaging material (SGS and MA only, $n = 45$) on colorimetric values ($L/a^*/b^* \pm SD$) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: L ($p_{groups} < 0.001$, $p_{storage} < 0.001$, $p_{interaction} = 0.395$, a^* ($p_{groups} < 0.004$, $p_{storage} = 0.147$, $p_{interaction} = 0.601$), b^* ($p_{groups} < 0.013$, $p_{storage} < 0.002$, $p_{interaction} = 0.250$). The different superscripts (ab) indicates the significant variation ($p < 0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

		Groups						
	Day	PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+MA	BioLB, SGS+MA	BioHB, SGS+MA	p-value ¹
L*	Initial*	59.41±0.40	59.41±0.40	59.41±0.40	59.41±0.40	59.41±0.40	59.41±0.40	
	10	56.56±0.34 ^a	58.66±0.83 ^{ab}	58.79±1.76 ^{ab}	59.46±1.06 ^b	58.63±0.36 ^{ab}	60.16±0.47 ^b	0.011
	20	59.39±1.23	60.51±0.68	60.38±2.55	60.56±1.37	58.69±1.10	61.87±1.12	0.213
a*	Initial*	12.77±1.91	12.77±1.91	12.77±1.91	12.77±1.91	12.77±1.91	12.77±1.91	
	10	14.52±0.59 ^a	18.11±0.76 ^b	17.25±1.15 ^b	17.47±1.00 ^b	16.78±0.34 ^b	18.22±0.34 ^b	<0.001
	20	15.48±1.40	16.77±0.08	16.66±2.08	16.46±0.65	15.37±0.61	17.88±0.15	0.126
b*	Initial*	10.90±2.37	10.90±2.37	10.90±2.37	10.90±2.37	10.90±2.37	10.90±2.37	
	10	11.68±1.41 ^a	14.90±0.80 ^b	14.11±1.55 ^{ab}	15.34±0.91 ^b	13.81±0.57 ^{ab}	16.40±0.11 ^b	0.002
	20	15.94±0.66	16.70±0.36	16.27±1.42	16.08±0.90	14.33±1.67	17.08±0.10	0.079

*Day 0, raw material before packaging ($n = 9$)
¹Significance level $p < 0.05$, GLM Multivariate

Significance between groups with and without SGS-treatment (L, a*, b*)			Significance between packaging materials regardless of SGS-treatment (colour)					
Day		p-value ¹	Day	Parameter	Packaging material		p-value ¹	
10	L	0.026	10	L	PA/PE	BioLB	BioHB	0.190
	a*	0.211		a*	PA/PE	BioLB	BioHB	0.066
	b*	0.043		b*	PA/PE	BioLB	BioHB	0.229
20	L	0.723	20	L	PA/PE	BioLB	BioHB	0.239
	a*	0.665		a*	PA/PE	BioLB	BioHB	0.137
	b*	0.434		b*	PA/PE	BioLB	BioHB	0.276

4.2.6 Texture

The resistance strength (N) was not affected by the experimental groups (GLM, $p=0.678$, Table 7), nor was it affected as a function of storage time (GLM, $p=0.844$). Consequently, there was not observed any interaction between groups and storage (GLM, $p=0.364$). Experimental groups with biobased material with traditional MA packaging had a slightly higher resistance strength compared to control group PA/PE MA at the end of storage time, but the differences remained insignificant (GLM, $p=0.608$). A similar trend was observed between the SGS-treated biobased groups compared to control group PA/PE SGS+MA. The biobased packaging materials were comparable to PA/PE material throughout storage time (GLM, $p>0.191$), as well as SGS-treatment did not differ from traditional MA packaging in any significant way (GLM, $p>0.492$).

Breaking force (N) was not affected by the experimental groups (GLM, $p=0.681$, Table 7), but for the most part, an increasing strength was needed to break the surface as a function of storage time (GLM, $p<0.013$), along with differences amongst the experimental groups at day 10 (GLM, $p<0.031$). BioHB SGS+MA needed significantly less strength to break the surface compared to control group PA/PE SGS+MA on day 10, but the difference was found insignificant at day 20 (GLM, $p=0.193$). The control groups with PA/PE material needed, on average, more strength to break the surface compared to both BioLB and BioHB on day 10 (GLM, $p<0.004$), suggesting samples with biobased material was softer. There was observed no difference between SGS-treated groups and traditional MA packaging, indicating that SGS-treatment did not give any further advantages (GLM, $p>0.259$).

The firmness (N) was also not affected by the experimental groups (GLM, $p=0.434$, Table 7), and it was also not affected as a function of storage time (GLM, $p=0.531$). Although, there was observed an interaction between experimental groups and storage time (GLM, $p<0.010$). Experimental groups with BioLB material had a significantly softer firmness than control groups with PA/PE material on day 10 (GLM, $p<0.015$), but this was found insignificant at the end of storage. Experimental groups with BioHB material had results that were comparable with control PA/PE material throughout storage. SGS-treatment remained comparable with MA packaging (GLM, $p>0.182$).

Table 7: Main effects of packaging material (SGS and MA only, n=39) on textural parameters (RF/BF/F (N) ± SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: RF ($p_{groups}=0.678$, $p_{storage}=0.844$, $p_{interaction}=0.364$), BF ($p_{groups}=0.681$, $p_{storage}<0.013$, $p_{interaction}<0.009$), F(60%) ($p_{groups}=0.434$, $p_{storage}=0.531$, $p_{interaction}<0.010$). The different superscripts (^{ab}) indicates the significant variation ($p<0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

		Groups						
	Day	PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+MA	BioLB, SGS+MA	BioHB, SGS+MA	p-value ¹
Resistance Force (N)	Initial*	7.594±0.31	7.594±0.31	7.594±0.31	7.594±0.31	7.594±0.31	7.594±0.31	
	10	11.451±2.65	9.548±1.65	9.788±2.16	10.332±0.59	11.094±0.14	8.685±0.67	0.349
	20	7.006±1.93	11.377±5.96	9.718±3.28	9.069±1.92	12.259±5.15	10.376±3.57	0.608
BF (N)	Initial*	9.934±1.57	9.934±1.57	9.934±1.57	9.934±1.57	9.934±1.57	9.934±1.57	
	10	13.245±2.0 ^{ab}	10.602±1.22 ^{ab}	11.041±2.32 ^{ab}	13.781±1.03 ^b	11.702±0.81 ^{ab}	9.567±0.43 ^a	0.031
	20	7.625±3.71	10.756±3.49	8.555±1.69	7.780±1.03	12.084±3.51	11.885±0.30	0.193
Firmness %	Initial*	7.001±0.33	7.001±0.33	7.001±0.33	7.001±0.33	7.001±0.33	7.001±0.33	
	10	9.345±1.43	7.781±1.01	8.374±1.89	11.313±1.49	7.916±0.64	8.563±1.01	0.054
	20	7.058±1.86	8.506±3.04	7.362±2.61	6.471±0.67	10.476±3.42	11.095±1.25	0.156

*Day 0, raw material before packaging (n=6)

¹Significance level $p<0.05$, GLM Univariate

Significance between groups with and without SGS-treatment			Significance between packaging materials regardless of SGS-treatment					
Day		p-value ¹	Day	Parameter	Packaging material			
p10	RF (N)	0.782	10	Resistance Force (N)	PA/PE	BioLB	BioHB	0.219
	BF (N)	0.955		Breaking Force (N)	PA/PE ^b	BioLB ^a	BioHB ^a	0.004
	Firmness (N)	0.342		Firmness (N)	PA/PE ^b	BioLB ^a	BioHB ^{ab}	0.015
20	RF (N)	0.492	20	Resistance Force (N)	PA/PE	BioLB	BioHB	0.191
	BF (N)	0.259		Breaking Force (N)	PA/PE	BioLB	BioHB	0.074
	Firmness (N)	0.182		Firmness (N)	PA/PE	BioLB	BioHB	0.148

5. Discussion

The usage of conventional plastic materials derived from petroleum feedstock is an industry well established in today's society, while the introduction of biobased materials to the global market is still in its early stages (Peelman et al., 2015). Limitations associated with renewable biopolymers are related to cost, material processing and performance in terms of maintaining product quality (Petersen et al., 1999). This thesis focuses entirely on the biobased material's performance and its effect on Atlantic salmon during storage, using petroleum-based PA/PE material as a control. To measure the effect, two packaging methods were performed, in addition to SGS-treatment as an experimental factor. Each packaging method was divided into two experiments, of which there were different raw materials.

It is relevant to mention some of the structural properties of the materials to understand each performance. The heat resistance has a significant correlation to the crystallinity of a material, whereas both temperature resistance and amount of crystallinity increases and decreases along with each other (Peelman et al., 2015). When it comes to the material's barrier properties and overall material structure in this study, BioLB was the most bendable and flexible. It was the least acceptable for high temperature while sealing, since the outer part of the edge sometimes melted off during the process. The exact temperature of the chamber machine is not given, but the holding strength was 1.2 seconds (Webomatic support, 2021). According to the datasheet of both BioLB and BioHB, the chamber machine's holding strength was longer than the relative holding strength of the materials (1.0 and 0.5 seconds, respectively). However, the sealing itself was successful and completely tight for both biobased materials. The BioLB material was also less rigid than BioHB, suggesting it has a lower crystallinity. Ebnesajjad (2013) reported that a higher degree of crystallinity can give higher barrier properties for a material, which makes sense according to BioHB having a higher barrier than BioLB. BioHB material contains cellulose and BioPBS, whereas the latter is proven to show excellent thermoplastic processability along with having high crystallinity (Nilsen-Nygaard et al., 2021).

MA packaging was performed by placing the CPET tray with samples inside each vacuum pouch before being filled with the MA and sealed. It was contemplated whether or not submerging the package under water to analyse the buoyancy force using a texture analyser would be suitable to determine the exact g/p ratio inside each package. The buoyancy force has

previously been studied to be an effective method to measure both solubility of CO₂ and calculate the volume inside a package (Abel, 2021; Rotabakk et al., 2007). The reason for not doing so was that there was no top film layer sealed directly to the CPET tray, but rather the tray containing salmon was placed inside a vacuum pouch filled with MA. It was therefore impossible to conduct the measurements with the atmosphere-filled pouches because it would, due to the principle of buoyancy effect, be forced towards the water surface during submergence. Consequently, it was only assumed that the *g/p* ratio was 4:1±5 %, but there is no certainty of the actual *g/p* ratio throughout storage.

Each experiment's raw materials had different suppliers and were harvested at a different time of year. The outset opening of experiment one was with raw material that had been slaughtered that same day, whereas experiment two had a delay with the raw material being nine days post mortem. However, it is not inconceivable that the fish is filleted and distributed to consumers nine days after slaughter, if one takes into account the large export market Norway has. The development for export volume for salmon indicates to be in excess of 380k tonnes in 2021 (Norges sjømatråd, 2021). Although, comparing these raw materials is challenging due to the many variations. Unrelated populations from different fish farms can vary due to different feeding and farming strategies (Lerfall, Bendiksen, Olsen, Morrice, & Østerlie, 2016). Rotabakk et al. (2018) examined the effect of season, localisation of fish farms, filleting regime and storage time of Atlantic salmon, and discovered that all these variables had an impact on different quality aspects of the salmon. Nevertheless, this study limits the comparison of the different raw material used in this study's experiments, as the essence is to focus on the biobased packaging material's effect on salmon's quality during storage time.

Erickson, Ma, and Doyle (2015) examined plastic materials with different oxygen permeabilities for packaging of Atlantic salmon. The findings from that study were that although the initial headspace in MA samples were 75 % CO₂/ 25 % N₂, the O₂ level within the package increased up to 3-5 % for materials with poor oxygen permeability. The BioHB material is commercially sold by Grounded Packaging as a provider of high barrier properties, compared to their standard vacuum pouch (BioLB). Even though the technical datasheet for the BioLB material claimed to have an OTR value of 0 cc/m²/24hrs (Appendix C.1), the observations from experiment two showed that BioLB had a significantly increased O₂ level compared to the control groups with PA/PE material. Erickson et al. (2015) also stated that the O₂ level reached its equilibration of dissolved O₂ in the fish muscle at 3-5 %, whereas this study

had similar equilibration levels for BioLB material at 2 % for traditional MA packaging at day five, and 3 % for SGS-treated samples at day 10 (Figure 14; Appendix B.4). If compared to the microbial analysis from experiment two, the TVC level reached its stationary phase around 8.5-9 log CFU/g for both BioLB materials at day 10 (Figure 15). Several studies have reported that if aerobic microbial activity utilizes more O₂ than the packaging material is able to diffuse into the package, the O₂ concentration will consequently decrease as a function of storage time (Fletcher, Summers, Corrigan, Cumarasamy, & Dufour, 2002; Ray & Bhunia, 2013; Rotabakk, Birkeland, Lekang, & Sivertsvik, 2008). The same trend is observed in the present study, whereas the TVC level reaches the stationary phase approximately at the same time as the O₂ level starts to drop in BioLB materials. BioHB was found to exceed above the control groups of PA/PE material as for prohibiting O₂ to permeate into the package, and remained significantly comparable throughout storage (Appendix B.4). The O₂ barrier properties for the biobased materials reflect over to the barrier properties for CO₂, whereas the BioLB material was not suitable to contain acceptable CO₂ levels through storage. The CO₂ concentration decreased rapidly in the BioLB material, while the BioHB material was significantly equal to the control groups with PA/PE material (Figure 14, Appendix B.4). The concentration gradient theory based on Fick's law (Majid Hassanizadeh & Leijnse, 1995), along with the fractions of gas composition in normal atmosphere (described in 2.4.2), can be used as an explanation to why the CO₂ level permeated at a higher rate than O₂ in the BioLB material. The CO₂ level inside the package at initial start was 58.6±0.7 %, compared to the CO₂ level on the outside of the package (approximately 0.03 %). This will cause a more rapid diffusion from high concentration through a polymer film with poor CO₂ barriers, compared to the ability for the smaller amount of O₂ level in normal atmosphere (approximately 20.9 %) to diffuse into the package with an O₂ level of 0 %. As mentioned previously, the permeability of a material is highly dependent on the structure and composition of the polymer. During extended storage, gasses will diffuse somewhat through the material for most packaging materials (Abel, 2021). Siracusa (2012) reported that a material with high barrier properties will reduce the amount of diffusion of gas to a minimum with storage temperature of 4 °C, as both BioHB and PA/PE did.

According to PFMG, the TVC level throughout the shelf-life of raw fish is acceptable with a maximum level of 7 log CFU/g (Bell, 1999). With respect to that maximum level, every experimental group from both experiments (one and two) were observed at unacceptable levels (>7 log CFU/g) at sampling day 10 (Figure 12, Figure 15). One exception was BioHB SGS+vacuum, which had a TVC of 6.5±0.5 log CFU/g in the wake of being the only

experimental group with no microbial growth on day five (Figure 15, Appendix A.1). Abel et al. (2020) found SGS-treatment prior to packaging as a potential alternative to give microbial inhibition and a longer shelf-life for salmon. The experimental groups with SGS-treatment from experiment one had only significantly lower TVC counts on day five compared to traditional VP. The groups from experiment one, in general, showed more stabilisation from day 10 onwards, even though they were significantly different from each other (Figure 15, Appendix A.1). BioLB SGS+vacuum showed a significantly higher microbial growth on sampling day 20 compared to the control group PA/PE SGS+vacuum, which can either be contradictory to the findings of the former mentioned study (Abel et al., 2020), or simply because the material itself has poor barrier properties. The same trend was observed in experiment two with MA packaging, where BioLB SGS+MA had significantly higher microbial growth than control group PA/PE SGS+MA (Appendix B.1). BioHB SGS+vacuum and BioHB SGS+MA were both significantly comparable to the control groups throughout storage time (Appendix A.1 and Appendix B.1, respectively), suggesting that BioHB material is as suitable as petroleum-based material for inhibiting microbial growth for both packaging methods. Vytejšková et al. (2017) examined PBS based packaging materials and its effect on poultry meat, and reported that even though the chemical, physical and mechanical properties of PA/PE and PBS-films are not similar, there was no significant limitation between them in practical applications for packaging of poultry meat. This relates well with BioHB, which consists of BioPBS, and the results in this study. Nevertheless, TVC does not refer to spoiled salmon in all cases, even though there is a certain relation between the total amount of microorganisms and the degree of spoilage (Gram & Huss, 1996). It might have been more informative to conduct methods for detection of SSO to see the relation between the actual spoilage organisms and other parameters conducted in this study. The ratio between SSO present in a product and the total amount of microorganisms can be substantial, and a product might not be entirely spoiled even though TVC accounts for it to be (Macé et al., 2013).

The pH level in fish stored in a MA can decrease in compliance with CO₂ incorporated into the fish, since dissolved CO₂ will chemically convert into carbonic acid (HCO₃⁻) in the muscle tissue (Chan et al., 2021; Dixon & Kell, 1989; Lee, Yang, Lin, & Chow, 1998; Sivertsvik et al., 2002). Nevertheless, several authors have reported similar observations about increased CO₂ concentration in the headspace atmosphere, leading to decreased pH (Lannelongue, Hanna, Finne, Nickelson, & Vanderzant, 1982; Manju, Jose, Srinivasa Gopal, Ravishankar, & Lalitha, 2007). If one factors out samples from BioLB material in experiment two, which had the most

insufficient barrier properties for MA packaging, samples from both BioHB and PA/PE had pH levels around 6 at the end, which is a decrease from a starting point of pH 6.31 ± 0.04 (Table 5). The possibility of CO_2 being converted into HCO_3^- , might be one of the explanations for decreased pH level in this study. Decomposition of nitrogenous compounds in post-mortem changes can lead to a production of TVB-N which can result in an increased pH level in the muscle tissue of the fish during storage due to the high pH level of the volatile base (Fidalgo et al., 2019; Manju et al., 2007). The pH level among all experimental groups in experiment one slightly increased from raw material (pH 6.14 ± 0.01) to the end of storage, except for BioHB vacuum, which had a decrease (pH 6.07 ± 0.05) (Table 1). TVB-N might explain the increase of pH during storage, but analysis for detection of TVB-N was not assessed in this study. The SGS-treatment in both experiments (one and two) were not significantly different from traditional VP or MA packaging (Table 1 and Table 5, respectively). One exception was for sampling day 10 in experiment one (GLM, $p < 0.014$), whereas BioHB SGS+vacuum and control group PA/PE SGS+vacuum were comparable to each other, yet had a higher pH level than the other experimental groups. Samples with BioLB material had a significantly higher pH level than control groups with PA/PE throughout experiment two, while the BioHB material was comparable to the control PA/PE material.

Experimental groups with traditional VP from experiment one had significantly lower drip loss (%) than the SGS-treated groups, whereas both BioLB+vacuum and BioHB+vacuum were comparable to the results of control group PA/PE+vacuum (Figure 13, Appendix A.3). The average amount of drip loss for all VP groups on day 10 (3.5 ± 0.75 %) aligns well with a previous study where conventional vacuum-packed salmon stored at 5°C had a 3.8 ± 1.3 % drip loss on storage day 15 (Fidalgo et al., 2020). The significantly higher drip loss observed for SGS-treated groups in experiment one is most likely caused by the amount of dissolved CO_2 in the muscle of the fish. This trend has been observed in several studies, where high CO_2 concentration during storage has proven to increase the drip loss (Sun et al., 2017; Zhang, Wang, Li, Li, & Xu, 2015). BioHB SGS+vacuum exceed above control group PA/PE SGS+vacuum with a significantly lower drip loss at both sampling days, while BioLB SGS+vacuum was equal to the control group at day 20 (Appendix A.3). CO_2 -enriched headspace has been reported to increase drip loss even more if the concentration is greater than 60 % in packaging of fatty fishes (Church & Parsons, 1995). Therefore, results from experiment two are also as expected since the SGS-treated groups had a significantly higher drip loss than MA packages infused with 60 % CO_2 (Figure 16, Appendix B.3). The biobased materials were

insignificant to the control groups with PA/PE, and gave comparable results throughout storage time (Appendix B.3). However, increased amount of CO₂ has also been reported by several authors to have the opposite effect on drip loss, causing it to decrease with increasing storage time (Rotabakk et al., 2008; Sivertsvik & Birkeland, 2006), which was not observed in this study.

Daskalova (2019) reports that salmon subjected to CO₂ during storage will lead to increased redness and yellowness. Contrariwise, SGS-treatment for experimental VP groups from experiment one were not significantly redder or more yellow than traditional VP. Although, every experimental group from both experiments (one and two), had increasing values for all colorimetric properties with increasing storage time (Table 2 and Table 6, respectively). A previous study suggested that salmon stored in biobased materials had a higher intensity of fresh colour compared with materials derived from petroleum-based resources (Pettersen, Bardet, Nilsen, & Fredriksen, 2011). This was not observed in this study, as the biobased materials had comparable results as the control group with PA/PE material. An increasing yellowness (b*) during storage time has previously been linked to lipid oxidation (Ruff, FitzGerald, Cross, & Kerry, 2002; Ruff, FitzGerald, Cross, Teurtrie, & Kerry, 2002). Although the experiments were designed to retard oxidation, a possible explanation for the initial b* value for experiment two (10.90±2.37) being higher than experiment one (7.59±1.83) could be that the raw material from experiment two was exposed to O₂ in a higher degree during the nine days prior (Table 2 and Table 6, respectively). Another factor can be that the unrelated fish farms use different feeding and farming strategies, which can affect the colourimetric perception of the fish (Lerfall et al., 2016).

On average, the highest textural values for VP from experiment one were observed on sampling day 10, when the raw material was 10 days post mortem. BioHB SGS+vacuum had least firmness that day, compared to control group PA/PE SGS+vacuum which was the firmest (Table 3). Samples with BioLB material was perceived as softer than control groups with PA/PE material in experiment two at sampling day 10 (Table 7). The softness observed with BioHB material (experiment one) and BioLB material (experiment two) is not associated with good textural properties, and is a quality aspect that is undesirable for the industry (Hultmann & Rustad, 2004). In experiment two, the breaking force observed on sampling day 10 for the biobased materials were significantly lower than the groups group with PA/PE (Table 7). Several authors have been reporting that softening of fillets due to the breakage of cross-links

and dissolving of collagen fibrils cause a decreased breaking force during storage (Ando, Nishiyabu, Tsukamasa, & Makinodan, 1999; Bremner & Hallett, 1985; Montero & Borderias, 1990). Since every experimental group from experiment two had the same raw material, the differences amongst the packaging material is assumed to be an explanation to it. The BioLB material had poor barrier properties, as previously stated, and there has been reported that high microbial growth (of aerobic bacteria) can lead to oxidation of lipids and further lead to changes in textural properties (Han, Ruiz-Garcia, Qian, & Yang, 2018). The overall textural properties are known to decrease during storage (Espe et al., 2004), and several factors can influence the changes, such as seasonal variations of collagen composition (Bjørnevik, Espe, Beattie, Nortvedt, & Kiessling, 2004), and the correlation with WHC and drip loss (Jittinandana, Kenney, Slider, & Kiser, 2002).

A misreading of the method in making supernatants for the HPLC analysis for detecting ATP and its degradation products may have affected the results of the H-values in experiment one. With regard to the method done by Lerfall, Bjørge Thomassen, and Jakobsen (2018) perchloric acid has been intentionally switched by TCA in this study. A different column was supposed to be used, but this was also intentionally altered. The misreading of the method was that there was supposed to be added 1.0ml of KOH (1.0 M) between the homogenisation of the sample and TCA, and the centrifugation. The pH was therefore not adjusted sufficiently according to the method. This was discovered after all 220 samples were analysed. Due to limited time to redo the analysis, the initial results remained included in this study. The degree of effect this had on the results is unknown, but their validation cannot be accurate. Although, it is interesting to see that the H-value was affected by the experimental groups and showed differences amongst the groups on sampling day 15 and 20 after a substantial storage time (Table 4). BioHB vacuum had a much higher H-value than control group PA/PE vacuum, while BioLB SGS+vacuum had a much higher H-value than PA/PE SGS+vacuum. Contradictory, BioLB vacuum had the lowest H-value on day 20 of the experimental groups with traditional VP, while BioLB SGS+vacuum had the highest H-value on day 20 out of all SGS-treated groups. A possible explanation might be seen according to the microbial growth during storage. There was found a significant correlation between H-value (%) and the TVC (log CFU/g) on sampling day 10, 15 and 20 ($r=0.658$, $r=0.643$, $r=0.699$; $p<0.01$, respectively), which indicate that the H-value can be explained accordingly to the development of TVC for those three sampling days. BioLB vacuum had the lowest H-value ($49\pm 17.9\%$) and the lowest TVC (7.5 ± 0.22 log CFU/g) out of all groups with traditional VP groups on day 20 (Table 4, Appendix A.1). Additionally,

BioLB SGS+vacuum also had the highest H-value (75.7 ± 9.74 %) and highest TVC (8.2 ± 0.16 log CFU/g) out of the SGS-treated groups at day 20. Hansen, Gill, Røntved, and Huss (1996) performed a study showing that the concentration of Hx increased with storage days, but increased more rapidly with a higher concentration in samples with higher microbial growth than the samples with less microbial growth. Another study observed that fresh fish stored at 4 °C had a reduced production of Hx, and made the conclusion that microorganisms were partly responsible for Hx production (Fletcher & Statham, 1988).

6. Conclusion

This thesis aimed to compare biobased and biodegradable packaging materials to a conventional petroleum-based material to see their effect on quality and shelf-life to fresh Atlantic salmon fillets. The objective was to maintain the quality and shelf-life to fresh salmon fillets during 20 days of storage (4 °C). Both VP and MA packaging was investigated as separate experiments, including SGS as an experimental factor to investigate any further advantages.

During storage with MA packaging, the poor barrier properties for BioLB were accentuated and may explain why it gave unsatisfactory results in both experiments, especially related to the quality parameters TVC, drip loss, texture, and H-value. The barrier properties for BioHB were acceptable throughout storage time and gave the most comparable product quality to control groups with PA/PE material. SGS-treatment gave some expected results, such as increased drip loss, lower TVC, and some effect on pH level, but there was overall a more considerable distinction between the packaging materials during this study.

Based on all analyses conducted in this study, it can be concluded that BioLB is not a suitable material for MA packaging. However, the results from VP can indicate that BioLB is somewhat suitable, even though the control group of PA/PE exceeded above. Contrastingly, the high barrier properties in BioHB gave acceptable results for maintaining quality for Atlantic salmon for both MA packaging and VP and is a suitable biobased and biodegradable packaging material for Atlantic salmon.

7. Future perspectives

This study has shown that it is possible to maintain the quality and shelf-life of Atlantic salmon by using biobased and biodegradable packaging material with high barrier properties. It would have been interesting to measure precise values for OTR, CO₂TR and WVTR for each plastic material to gain a broader understanding of how each material's barrier properties affect the product. Further investigation of each material's climate footprint would have raised the quality of the arguments for replacing the petroleum-based material with a biobased and biodegradable one. A Life Cycle Assessment would be of interest, and further work on other types of available biobased materials and their effect on the product quality of seafood should be researched.

The precise *g/p* ratio during MA packaging was not possible to detect because the trays were surrounded by vacuum pouches and prevented analysis of the buoyancy effect. Therefore, it would be beneficial to repeat the second experiment using biobased trays sealed with a biobased top film. It would also be beneficial in terms of measuring the CO₂ concentration compared to dissolved CO₂ during storage .

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Appendices

- Appendix A.1:** Experiment One: Results of Total TVC (log CFU/g)
- Appendix A.2:** Experiment One: Development of pH Level
- Appendix A.3:** Experiment One: Results of Drip Loss (%)
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Appendix A.1: Experiment One: Results of Total TVC (log CFU/g)

Table 1: Main effects of packaging material (SGS and vacuum only, n=108) on TVC (log CFU/g ± SE) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment one). GLM: $p_{groups} < 0.001$, $p_{storage} < 0.001$, $p_{interaction} < 0.001$. The different superscripts (abc) indicates the significant variation ($p < 0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

TVC (log CFU/g)				Groups			p-value ¹
Day	PA/PE vacuum	BioLB vacuum	BioHB vacuum	PA/PE SGS+ vacuum	BioLB SGS+ vacuum	BioHB SGS+ vacuum	
Initial*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
5	5.793±0.32 ^b	6.013±0.16 ^b	6.13±0.58 ^b	4.759±1.07 ^b	5.037±0.20 ^b	n.d. ^a	<0.001
10	7.343±0.32 ^{bc}	6.953±0.12 ^{ab}	7.877±0.21 ^c	7.043±0.20 ^{ab}	7.523±0.28 ^{bc}	6.520±0.48 ^a	0.002
15	7.523±0.18 ^{ab}	7.480±0.56 ^{ab}	7.833±0.09 ^{ab}	7.110±0.32 ^{ab}	8.080±0.10 ^b	6.743±0.73 ^a	0.020
20	7.627±0.12 ^{bc}	7.537±0.36 ^b	7.637±0.22 ^{bc}	6.787±0.14 ^a	8.153±0.16 ^c	6.860±0.19 ^a	<0.001

*Day 0, raw material

¹Significance level $p < 0.05$, GLM Univariate

Significance between groups with and without SGS-treatment		Significance between packaging materials regardless of SGS-treatment				
Day	p-value ¹	Day	Packaging material			p-value ¹
5	0.004	5	PA/PE	BioLB	BioHB	0.114
10	0.135	10	PA/PE	BioLB	BioHB	0.988
15	0.274	15	PA/PE	BioLB	BioHB	0.256
20	0.182	20	PA/PE	BioLB	BioHB	0.048

Appendix A.2: Experiment One: Development of pH Level

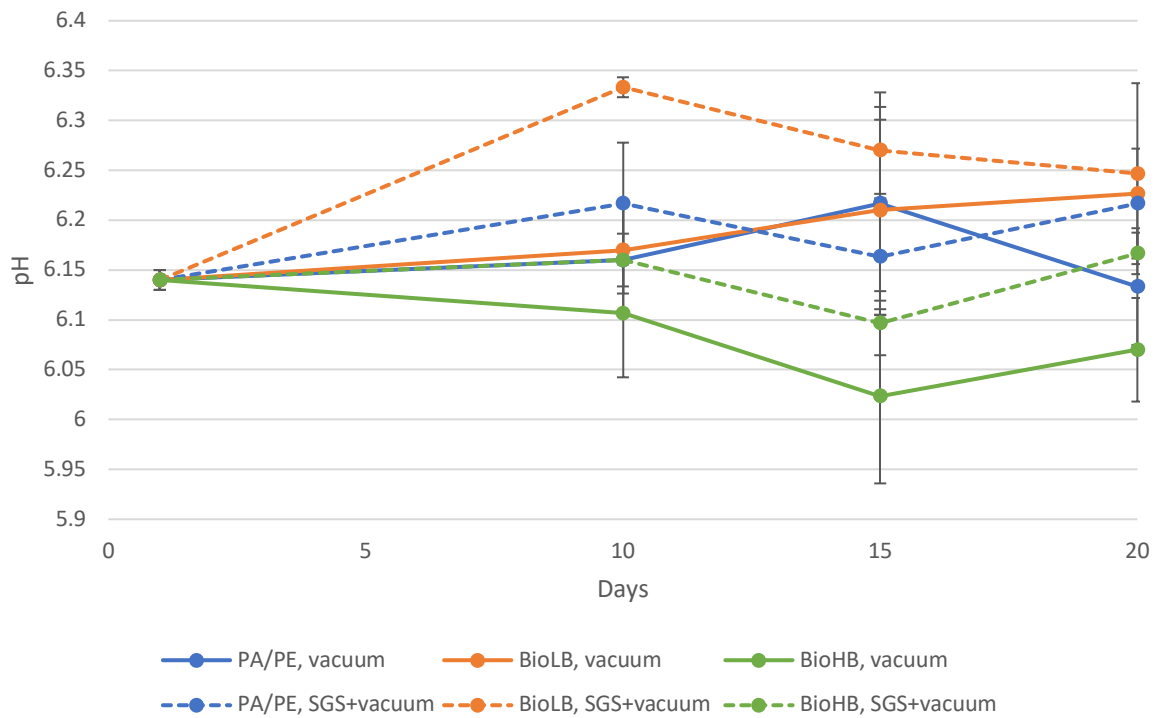


Figure 1: Main effects of packaging material (SGS and vacuum only, $n=60$) on pH values ($\pm SD$) during 20 days of storage ($4^{\circ}C$) of fresh Atlantic salmon (experiment one). GLM: $p_{groups} < 0.001$, $p_{storage} = 0.409$, $p_{interaction} = 0.310$.

Appendix A.3: Experiment One: Results of Drip Loss (%)

Table 2: Main effects of packaging material (SGS and vacuum only, n=36) on drip loss (% \pm SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment one). GLM: $p_{groups} < 0.001$, $p_{storage} < 0.001$, $p_{interaction} < 0.010$. The different superscripts (^{abc}) indicates the significant variation ($p < 0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

Drip loss %		Groups					p-value ¹
Day	PA/PE vacuum	BioLB vacuum	BioHB vacuum	PA/PE SGS+ vacuum	BioLB SGS+ vacuum	BioHB SGS+ vacuum	
Initial*	0	0	0	0	0	0	
10	3.12 \pm 0.37 ^a	4.36 \pm 0.59 ^{ab}	3.02 \pm 0.40 ^a	8.83 \pm 1.37 ^c	6.49 \pm 0.37 ^{bc}	4.69 \pm 1.68 ^{ab}	<0.001
20	5.31 \pm 1.31 ^a	5.68 \pm 0.05 ^a	4.41 \pm 0.74 ^a	7.68 \pm 0.20 ^b	7.64 \pm 0.69 ^b	5.48 \pm 0.53 ^a	<0.001

*Day 0, raw material before packaging

¹Significant level $p < 0.05$, GLM Univariate

Significance between groups with and without SGS-treatment		Significance between packaging materials regardless of SGS-treatment			
Day	p-value ¹	Day	Packaging material		p-value ¹
10	<0.001	10	PA/PE	BioLB	0.246
20	0.002	20	PA/PE	BioLB	0.051

Appendix B.1: Experiment Two: Results of Total TVC (log CFU/mg)

Table 3: Main effects of packaging material (SGS and MA only, n=108) on TVC (log CFU/g ± SE) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: $p_{\text{groups}} < 0.001$, $p_{\text{storage}} < 0.001$, $p_{\text{interaction}} < 0.001$. The different superscripts (^{abc}) indicates the significant variation ($p < 0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

Day	Groups						p-value ¹
	PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+MA	BioLB, SGS+MA	BioHB, SGS+MA	
Initial*	3.85±0.06	3.85±0.06	3.85±0.06	3.85±0.06	3.85±0.06	3.85±0.06	
5	5.873±0.22 ^{ab}	6.047±0.08 ^{ab}	5.827±0.53 ^{ab}	6.250±0.09 ^b	6.063±0.28 ^{ab}	5.470±0.05 ^a	0.054
10	7.530±0.17 ^a	8.933±0.13 ^b	7.110±0.24 ^a	7.120±0.32 ^a	8.433±0.07 ^b	7.163±0.21 ^a	<0.001
15	7.397±0.32 ^b	8.980±0.16 ^c	6.780±0.15 ^a	7.197±0.08 ^{ab}	8.827±0.19 ^c	7.063±0.19 ^{ab}	<0.001
20	7.783±0.07 ^b	8.827±0.05 ^c	7.246±0.12 ^{ab}	7.470±0.52 ^{ab}	8.570±0.19 ^c	7.007±0.08 ^a	<0.001

*Day 0, raw material

¹Significance level $p < 0.05$, GLM Univariate

Significance between groups with and without SGS-treatment		Significance between packaging materials regardless of SGS-treatment				
Day	p-value ¹	Day	Packaging material		p-value ¹	
5	0.941	5	PA/PE	BioLB	BioHB	0.040
10	0.439	10	PA/PE ^a	BioLB ^b	BioHB ^a	<0.001
15	0.958	15	PA/PE ^b	BioLB ^c	BioHB ^a	<0.001
20	0.441	20	PA/PE ^b	BioLB ^c	BioHB ^a	<0.001

Appendix B.2: Experiment Two: Development of pH Level

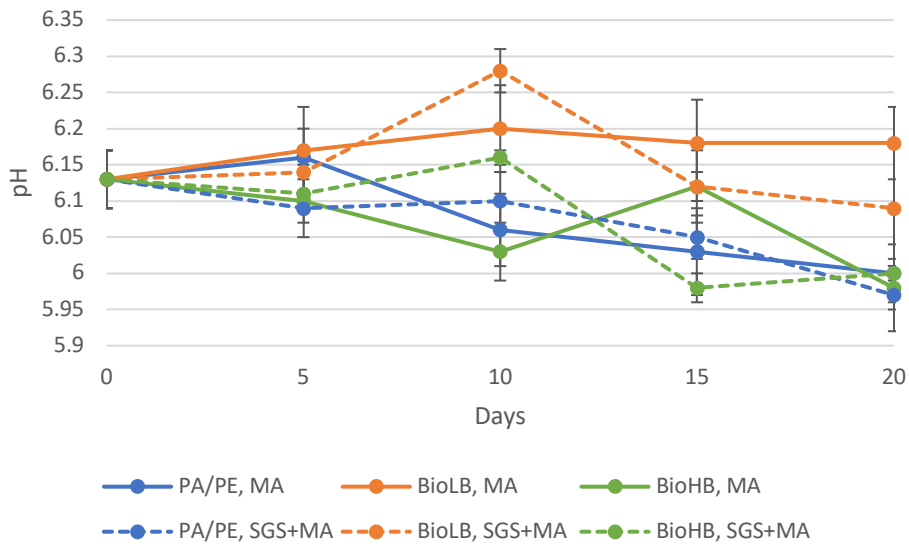


Figure 2: Main effects of packaging material (SGS and MA only, $n=60$) on pH values ($\pm SD$) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: $p_{groups} < 0.001$, $p_{storage} < 0.001$, $p_{interaction} < 0.001$

Appendix B.3: Experiment Two: Results of Drip Loss (%)

Table 4: Main effects of packaging material (SGS and MA only, n=36) on drip loss (%±SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: $p_{groups}=0.050$, $p_{storage}<0.020$, $p_{interaction}<0.781$. The different superscripts (^{abc}) indicates the significant variation ($p<0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

		Groups						
	Day	PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+ MA	BioLB, SGS+ MA	BioHB, SGS+ MA	p-value ¹
Drip	Initial*	0	0	0	0	0	0	
loss %	10	5.03±0.17	6.16±0.43	5.25±1.37	6.78±0.67	7.31±1.17	6.52±1.25	0.081
	20	7.12±1.21	6.86±2.30	5.87±1.41	8.08±0.34	7.60±0.87	7.18±0.72	0.465

*Day 0, raw material before packaging

¹Significant level $p<0.05$, GLM Univariate

Significance between groups with and without SGS		Significance between packaging materials regardless of SGS-treatment				
Day	p-value ¹	Day	Packaging material		p-value ¹	
10	0.006	10	PA/PE	BioLB	0.369	
20	0.102	20	PA/PE	BioLB	0.369	

Appendix B.4: Experiment Two: Headspace Gas Composition (CO₂ and O₂)

Table 5: Main effects of packaging material (SGS and MA only, n=90) on CO₂ (A) and O₂ (B) (%±SD) during 20 days of storage (4 °C) of fresh Atlantic salmon (experiment two). GLM: CO₂ ($p_{groups}<0.001$, $p_{storage}<0.001$, $p_{interaction}<0.001$), O₂ ($p_{groups}<0.001$, $p_{storage}<0.001$, $p_{interaction}<0.001$). The different superscripts (^{abc}) indicates the significant variation ($p<0.05$) between groups at the same sampling day by a one-way ANOVA and Tukey's comparison test.

CO ₂ %							
Day	Groups						p-value ¹
	PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+MA	BioLB, SGS+MA	BioHB, SGS+MA	
Initial*	58.6±0.70	58.6±0.70	58.6±0.70	58.6±0.70	58.6±0.70	58.6±0.70	
5	56.3±2.49 ^b	30.0±1.10 ^a	53.9±0.31 ^b	54.5±0.42 ^b	29.8±0.60 ^a	54.3±0.27 ^b	<0.001
10	57.0±0.25 ^c	13.7±1.80 ^a	53.7±0.60 ^b	54.3±0.31 ^b	14.2±1.27 ^a	54.3±0.56 ^b	<0.001
15	56.5±0.45 ^b	8.2±0.91 ^a	54.7±1.05 ^b	54.5±0.12 ^b	9.0±0.91 ^a	56.1±0.78 ^b	<0.001
20	57.1±0.31 ^{cd}	5.3±0.00 ^a	55.0±0.44 ^{bc}	54.3±0.17 ^b	5.8±0.90 ^a	56.1±0.40 ^{cd}	<0.001

O ₂ %							
Day	Groups						P-value ¹
	PA/PE, MA	BioLB, MA	BioHB, MA	PA/PE, SGS+MA	BioLB, SGS+MA	BioHB, SGS+MA	
Initial*	0	0	0	0	0	0	
5	0.53±0.83 ^a	2.0±0.13 ^b	0.02±0.00 ^a	0.06±0.01 ^a	1.8±0.08 ^b	0.02±0.00 ^a	<0.001
10	0.01±0.02 ^a	1.93±1.17 ^b	0 ^a	0.07±0.02 ^a	3.00±0.36 ^b	0.01±0.00 ^a	<0.001
15	0	0.52±0.46	0	0	0.36±0.42	0	0.088
20	0 ^a	0 ^a	0 ^a	0 ^a	1.34±1.17 ^b	0 ^a	0.024

*Day 0, raw material

¹Significance level $p<0.05$, GLM Univariate

Significance between groups with and without SGS-treatment			Significance between packaging materials regardless of SGS-treatment					
Day	Parameter	p-value ¹	Day	Parameter	Packaging material		p-value ¹	
5	CO ₂	0.929	5	CO ₂	PA/PE ^b	BioLB ^a	BioHB ^b	<0.001
	O ₂	0.644			PA/PE ^a	BioLB ^b	BioHB ^a	<0.001
10	CO ₂	0.958	10	CO ₂	PA/PE ^b	BioLB ^a	BioHB ^b	<0.001
	O ₂	0.551			PA/PE ^a	BioLB ^b	BioHB ^a	<0.001
15	CO ₂	0.995	15	CO ₂	PA/PE ^b	BioLB ^a	BioHB ^b	<0.001
	O ₂	0.727			PA/PE ^a	BioLB ^b	BioHB ^a	0.007
20	CO ₂	0.974	20	CO ₂	PA/PE ^b	BioLB ^a	BioHB ^b	<0.001
	O ₂	0.152			PA/PE	BioLB	BioHB	0.118

Appendix C.1: Technical Datasheet of Biobased and Biodegradable Material (Low Barrier) (BioLB)

13/11/2019

GROUND

Technical Data Sheet – Home Compostable Single Layer Film (HSF)

Product Type		Compostable Laminate		Product Description		Compostable Single Layer Film	
Specifications	ASTM	Metric Units	US Units	Metric Values	US Values		
Thickness		Micron (μ)	mil	78-82	3.1-3.2		
Yield		m^2/kg	in^2/lb	10.2	7171		
Density		g/cm^3	lb/in^3	1.25	0.045		
COF Kinetic Film/Film	D1894	-	-	0.20 - 0.40	0.20 - 0.40		
COF Kinetic Seal/Seal	D1894	-	-	0.20 - 0.40	0.20 - 0.40		
WVTR 38°C 90% RH (ASTM E96)		$g / m^2 / 24hrs$	$g / m^2 / 24hrs$	0	0		
OTR 23°C 0% RH		$cc / m^2 / 24hrs$	$cc / m^2 / 24hrs$	0	0		
Sealing temp. range (1. sec)		°C	°F	80 - 120	176 - 248		
Sealing Strength MD, @100°C	F2029	N/25mm	lbf/in	> 32	>7		
Tensile Strength @ Break							
MD	D882			20-30	2900-4350		
TD	D882	MPa		10-20	1450-2900		
Tensile Elongation							
MD		%		> 300	> 300		
TD		%		> 500	> 500		
Haze	D1003	%		< 31	< 31		
Light Transmittance	D1894	%		> 79	> 79		
Recommended Storage Instructions		Temp 17 -23°C, RH 35 – 55%. Avoid cold and keep away from direct sunlight and odours; the material is to be acclimatized in the production area ideally 24hrs before use. It is recommended to use the material within 4 Months.					
Food Contact		Declared as complying to food safety standards					

Figure 3: Technical datasheet for the biobased and biodegradable material with low barrier (Grounded Packaging, 2019).

Appendix C.2: Technical Datasheet of Biobased and Biodegradable Material (High Barrier) (BioHB)

13/11/2019

GROUNDING

Technical Data Sheet – Home Compostable Duplex Laminate Film (HDF)

Product Type	Compostable Laminate	Product Description	Compostable Duplex Laminate
Specification Number	HCFD2 (b) (HK1)	Product Structure	19µ NK / Ink / Adhesive / 30µ Sealant Web
Production Method	Print / Laminate / Slit	Approved Materials	All materials conform to EN13432
Vincotte OK Compost Accreditation Reference	S370 (0 15-1537-A)	Vincotte Seedling Accreditation Reference	7P2055-Ed.B

Specification	Tolerances		
	Min	Target	Max
Thickness	44.1	49	53.9
Yield	58.95	65.5	72.05
COF (A/A Dynamic)	0.20	0.30	0.40
WVTR 38°C 90% RH (ASTM E96)		< 14	g / m ² / 24hrs
OTR 23°C 0% RH		< 1	cc / m ² / 24hrs
Sealing Range (15psi 0.5sec dwell)	80	100	°C
Heat Sealing Strength		> 600	g / 25mm
Tensile Strength			
MD		71.46	
TD		34.60	N / mm ²
Elongation To Break			
MD		16	
TD		44	%
Tear Strength			
MD		750	
TD		1350	mN

Recommended Storage Instructions	Temp 17 -23°C, RH 35 – 55%. Avoid cold and keep away from direct sunlight and odours, the material is to be acclimatized in the production area ideally 24hrs before use. It is recommended to use the material within 4 Months.
Food Contact	

Figure 4: Technical datasheet for the biobased and biodegradable material with high barrier (Grounded Packaging, 2019).

Appendix C.3: Technical Datasheet of Petroleum-Based Material (PA/PE)



UŽDAROJI AKCINĖ BENDROVĖ

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PRODUCT INFORMATION AND DATA SHEET

No 13-7601

VAKPAK-TM film for packaging

Description:

VAKPAK - is a very transparent coextrusion PA/PE film. Designed for vacuum and modified atmosphere packaging. Can be used as lidding film.

Typical values:

Properties	Test method	Unit	Typical values			
Thickness	"Lietpak"	micron	80±5%	100±5%	120±5%	130±5%
Unit weight	"Lietpak"	g/m ²	77.4	96.8	116.2	125.8
Yield	"Lietpak"	m ² /kg	12.9	10.3	8.6	7.9
Heat sealing temperature	"Lietpak"	°C	140 - 160	150-170	150-170	150-170
O ₂ permeability (23°C – 75 % RH)	ASTM D-3985	cc/m ² ·24 h-	~52	~52	~27	~27
Tensile strength -machine direction	ISO 527-3	MPa	30	30	30	40
-cross machine direction			27	27	27	35
Permeability to: Water vapour	ASTM F 1770	cc/m ² ·24 hrs	2.3	1.7	1.5	1.4

Figure 5: Technical datasheet for PA/PE material (Lietpak, n.d.).

Appendix C.4: Technical Datasheet of Tray (CPET)

Generelt

Artikelnummer	C 2125-1A
Form	Rektangulær
Antal rum	1
Tilberedning	Ovn/Mikrobølgeovn
EAN	5703969004953

Gravure

Glas og gaffel	Ja
Genbrugspile	Ja
Kavitetsnr.	Ja
Grüne Punkt	Nej
Dybdeangivelse	Nej
Faerch artikelnummer	Ja
Logo	Ja
Kundelogo	Nej

Teknisk Info

Længde	125,3 mm
Tolerance længde	+/- 0,6 mm
Bredde	99,1 mm
Tolerance bredde	+/- 0,6 mm
Tolerance centrerung	+/- 0,9 mm
Dybde	32,5 mm
Tolerance dybde	+/- 0,6 mm
Stablehøjde	1,7 mm
Volume	230 ml
Nominal gauge	550 µm
Vægt i gram pr. emne	8,35 g
Tolerance vægt	+/- 10%

Materiale

Hovedstykliste	6812
Materiale	CPET
Farve	Sort
NIR detekterbar	Nej
Min. temp. (celsius)	-40 °C
Max. temp. (celsius)	220 °C

Forpakning

Forpakning	Karton
Forpakning længde	570 mm
Forpakningbredde	400 mm
Forpakning højde	225 mm
Forpakningvolum	0,051 m ³
Nettovægt (uden emballage)	12,00 kg
Vægt pr. forpakning	12,60 kg
Palletype	Engangspalle
Pallehøjde	1255 mm
Produkter pr. forpakning	1440
Kartoner pr. lag	4
Lag pr. palle	5
Kartoner pr. palle	20
Total produkter pr. palle	28800

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Figure 6: Technical datasheet for the CPET tray used in this study (Faerch, n.d.)

