Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

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The Effect of High-Pressure Processing and Soluble Gas Stabilization on Biobased Packaging Material

Packaging of Rehydrated Clip-Fish Loins

Master's thesis in Chemical Engineering and Biotechnology
Supervisor: Jørgen Lerfall
Co-supervisor: Sherry Stephanie Chan and Bjørn Tore Rotabakk
June 2023

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Nofima

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Preface

This Master's thesis marks the completion of the 5-year long master's program in Chemical Engineering and Biotechnology and covers 30 ECST points. The thesis is a continuation of a specialization project (TBT4500) carried out during the fall of 2022. Therefore, some parts of the theory and methodology are similar.

The thesis was completed in collaboration with Nofima AS, Stavanger, and the Department of Biotechnology and Food Science at NTNU, Trondheim. The experimental execution took place mainly at Nofima AS, Stavanger, over a period of two months, with some last analyses at NTNU Kalvskinnet.

I would like to thank everyone I got the opportunity to work with during my stay at Nofima Stavanger. Especially thanks to Mette Risa Vaka and Gorana Drobac for their help and support in the laboratory work. I would also like to express my gratitude to my supervisors Professor Jørgen Lerfall (NTNU), Postdoc Sherry Stephanie Chan (Nofima), and Scientist Bjørn Tore Rotabakk (Nofima) for their great support and feedback throughout the project.

And finally, I would like to express my gratitude to my family, friends, and my partner in life, Per Holt-Seeland. You have been my rock these last years, keeping my sanity in check.

Trondheim, 10th June 2023

Gøril Nygård Pettersen

Abstract

The objective of this Master's thesis was to evaluate if biodegradable food packaging (cellulose film laminated to BioPBS) offers the same level of protection as non-recyclable packaging (PA/PE) when exposed to soluble gas stabilization (SGS) and high-pressure processing (HPP). In addition, the study aimed to unveil any synergistic effects between HPP and SGS by combining them as hurdle technology. To determine the effect SGS and HPP had on the packaging, the quality and shelf life of rehydrated clip-fish were evaluated by measuring the microbial growth, drip loss, water- and protein content, protein denaturation, free amino acid distribution, as well as the surface color and the texture, over a period of 48 days.

The various quality analyses revealed minimal distinctions between the packaging options. The biodegradable and bio-based bags provided an equivalent level of protection when exposed to SGS and HPP as the non-recyclable PA/PE bags.

In regard to any synergistic effects between SGS and HPP. SGS and HPP combined extended the microbial shelf life of rehydrated clip-fish. HPP at 600 MPa with SGS resulted in the lowest microbial growth. However, HPP at 400 MPa combined with SGS gave similar results as HPP at 600 MPa without SGS. Proteins had already been denatured due to the salting and drying process of the clip-fish, and therefore no significant changes in the surface color of were detected after the HPP treatment on day 0. Textural properties varied, where HPP-treated clip-fish became firmer and SGS+HPP-treated clip-fish became softer. The free amino acid (FAA) content significantly differed, with SGS+HPP-treated clip-fish having the highest FAA content. Thus, SGS may have caused more FAA. If the FAA does cause off-flavor, the sensorial shelf life of the clip-fish may not reflect the microbial shelf life.

Even though there was a synergistic effect between SGS and HPP in regard to microbial growth, and the other quality parameters were within reasonable variations, it may have had the opposite effect in regard to FAA. This needs further investigation.

Sammendrag

Formålet med denne masteroppgaven har vært å evaluere hvorvidt biologisk nedbrytbar matemballasje (folie av cellulose laminert til BioPBS) gir like god beskyttelse som ikke-gjenvinnbar emballasje (PA/PE), når det utsettes for høytrykks behandling (HPP) og 100% CO_2 (SGS). I tillegg har oppgaven hatt som mål å avdekke potensielle synergiske effekter mellom HPP og SGS ved å kombinere dem som hindrings-teknologi. For å bestemme effekten av SGS og HPP på emballasjen ble klippfiskens kvalitet og holdbarhet evaluert ved å måle mikrobiell vekst, drypptap, vann- og protein innhold, proteindenaturering, frie aminosyrer, overflatefarge og tekstur, over en periode på 48 dager.

De ulike kvalitetsanalysene avdekket minimale forskjeller mellom emballasjealternativene. De biologisk nedbrytbare og biobaserte posene ga et tilsvarende beskyttelsesnivå når de ble utsatt for SGS og HPP, som de ikke-resirkulerbare PA/PE-posene.

Med hensyn til eventuelle synergistiske effekter mellom SGS og HPP, førte en kombinasjon av SGS og HPP til utvidet mikrobiologisk holdbarhet av den rehydrert klippfisk. Lavest mikrobiell vekst ble påvist ved 600 MPa HPP i kombinasjon med SGS. Imidlertid ga HPP ved 400 MPa kombinert med SGS lignende resultater som HPP ved 600 MPa uten SGS. Proteiner har allerede blitt denaturert på grunn av salt- og tørkeprosessen til klippfisken, og derfor ble det ikke oppdaget signifikante endringer i kilppfiskens farge etter trykkbehandlingen på dag 0. Teksturen varierte, der HPP-behandlet klippfisk ble fastere og SGS+HPP-behandlet klippfisk ble løsere. Det ble observert signifikante forskjeller i innholdet av frie aminosyrer (FAA), der SGS+HPP-behandlet klippfisk hadde høyest innhold av frie aminosyrer. Dermed kan SGS ha forårsaket mer FAA. Dersom FAA forårsaker bismak, vil den sensoriske holdbarheten til klippfisken potensielt ikke gjenspeile den mikrobielle holdbarheten.

Selv om det var en synergistisk effekt mellom SGS og HPP, med hensyn til mikrobiell vekst og de andre kvalitetsparametrene var innenfor rimelige variasjoner, kan det ha hatt motsatt effekt i forhold til FAA. Dette trenger ytterligere undersøkelser.

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Abbreviations

BioPBS	Biobased PolyBytulene Succinate
BOA	biaxially oriented polyamide
C	Control - 0.1 MPa in vacuum
CO_2	Carbon dioxide
CO2TR	carbon dioxide transmission rate
CS	Control - 0.1 MPa in vacuum with SGS
DL	Drip loss
DSC	Differential scanning calorimetry
EVA	polyethylene–vinyl acetate co-polymer
FAA	Free amino acid
GLM	General linear model
H400-B	HPP treatment at 400 MPa for 5 min and packaged in bio-based bags
H400-L	HPP treatment at 400 MPa for 5 min and packaged in PA/PE bags
H600-B	HPP treatment at 600 MPa for 5 min and packaged in bio-based bags
H600-L	HPP treatment at 600 MPa for 5 min and packaged in PA/PE bags
HPP	High-pressure processing / high-hydrostatic pressure treatment
HSPB	hydrogen sulphid producing bacteria count
L&H	Long & Hammer
LDPE	low-densitypolyethylene
MAP	Modified atmospheric packaging
NH_3	Ammonia
OTR	oxygen transmission rate
PA	Polyamide
PBS	Poly(butylene succinate)
PE	Polyethylene
PET	polyethylene terephthalate
PP	polypropylene
PVDC	polyvinylidene dichloride
SGS	Soluble gas stabilization
SH400-B	SGS+HPP treatment at 400 MPa for 5 min and packaged in bio based bags
SH400-L	SGS+HPP treatment at 400 MPa for 5 min and packaged in PA/PE bags

SH600-B	SGS+HPP treatment at 600 MPa for 5 min and packaged in bio based bags
SH600-L	SGS+HPP treatment at 600 MPa for 5 min and packaged in PA/PE bags
SSO	Specific spoilage organisms
SY	surlyn
TAPC	Total aerobic plate count
TMA	Trimethylamine
TMAO	Trimethylamine oxide
TPC	Total psychrotrophic count
WHC	Water holding capacity
WVTR	water vapor transmission rate
ww	wet weight

1 Introduction

The environmental impact of food packaging is a growing concern, mainly due to its high production volume, short usage time, and issues with waste management and littering. To align with the principles of the circular economy, reducing, reusing, recycling, and redesigning are vital strategies (Tsironi and Taoukis, 2018; Geueke et al., 2018). As we become more aware of sustainability issues, it is clear that promoting sustainable consumption and production patterns is crucial. This is one of the 17 UN Sustainable Development Goals and aims to create a better future for all by adopting sustainable practices. One concern that is being addressed is food waste, and suitable packaging is key to prevent this. According to the European Commission (n.d.), Europe generates 26 million tonnes of plastic waste annually, with plastic accounting for approximately 80% of marine litter (these figures do not solely refer to food packaging). Consequently, the EU is implementing measures to combat plastic pollution and marine litter, aiming to speed up the shift towards a circular and resource-efficient plastics economy. The European Commission also reports that 87% of Europeans are worried about the impact of plastic products on the environment. As a consequence, consumers become increasingly aware of the environmental impact caused by their purchasing habits. Thus, sustainable food packaging has become a primary concern for many companies. There are numerous choices to guarantee that food remains fresh and tasty while also being packaged in an environmentally friendly manner. Some popular sustainable packaging options include paper-based materials, plant-based plastics, and even reusable glass containers. By choosing sustainable food packaging, companies not only can reduce their environmental footprint but also appeal to consumers who are looking for eco-friendly options.

Norway has a rich history in the fishery industry. Aquaculture and fisheries are one of Norway's most earning export platforms, with 2.94 million tons of seafood exported in 2022, valued at 151.4 billion NOK. Aquaculture contributed 111.3 billion NOK, while fisheries added 40.1 billion NOK (Norwegian Seafood Council, 2023). Clipfish and stockfish were once the most important products exported and have been exported as early as the 1700s, with public records dating back to 1838 (Dokka, 2020). In 2022, Norway exported 85 174 tons of clipfish, earning 5.57 billion NOK (Norwegian Seafood Council, 2023). However, today salmon is Norway's top aquaculture export, with a recorded output of 1.31 million tons in 2022, valued at 110.8 billion NOK (Norwegian Seafood Council, 2023).

Fish muscle is easily perishable and easily influenced by extensive harsh treatments that can influence the product's quality. It is well known that high pressure and temperature will prevent microbial growth. However, these treatments can significantly influence the product's quality. Mild processing technologies such as low temperature, pasteurization, fermentation, ultra-sound processing, and soluble gas stabilization (SGS) are increasingly popular to prolong the shelf life of the fish product without negatively influencing its quality.

1.1 Objectives

In a project thesis carried out by the same author during the fall of 2022, the combination of SGS and high-pressure processing (HPP) at varying pressure levels (300 MPa, 400 MPa, and 600 MPa) were evaluated to determine any synergistic effect in prolonging the shelf life of rehydrated clip-fish. The findings revealed that the combination had a positive effect as long as the pressure level was not too high. At 600 MPa, the HPP effect overshadowed the SGS effect. At 400 MPa, clip-fish treated with SGS and HPP had significantly better microbiological shelf life than those only treated with HPP.

HPP and SGS are considered to be milder processing techniques. Thus, they are suitable for the fishery industry. However, there has not been much research on how these treatments can impact biodegradable and more sustainable food packaging materials. Therefore, the objective of this master's thesis is to determine the potential of using bio-based and biodegradable bags for food packaging when exposed to SGS and HPP. This will be determined by evaluating the quality and microbial shelf life of rehydrated clip-fish packed in two different packages (one biodegradable and one fossil-based), and treated with a combination of SGS and HPP (at 400 MPa and 600 MPa). In addition, the thesis aims to unveil any synergistic effects between HPP and SGS by combining them as hurdle technology, to reassure the conclusion of the project thesis.

2 Background

This Master thesis is a continuation of the Project thesis "Optimizing the quality and shelf life of rehydrated clipfish by combining high-pressure treatment with soluble gas stabilization" by the same author. The project aimed to evaluate the synergistic effect by combining SGS with HPP at different pressure levels (300 MPa, 400 MPa, and 600 MPa). Thus, some of the theory and methodology will be similar. The study concluded that there was a synergistic effect by combining SGS and HPP when the pressure level was not too high. At 600 MPa, the HPP effect surpassed the SGS effect. However, at 400 MPa, there was significantly higher microbial growth in the clip-fish treated with only HPP compared to the clip-fish treated with SGS and HPP, and no significant differences between the other measured quality parameters.

2.1 Hurdle technology

The number of spoilage organisms in a food product determines its microbiological shelf life, as explained in section 2.3.1. However, changes in sensory properties also affect the shelf life. The food industry constantly develops new technologies to extend shelf life while preserving freshness, color, texture, and nutrients. The term hurdle technology is used when several less invasive technologies are used to preserve a food product and prolong its shelf life. This could for example be a combination of modified atmospheric packaging (MAP), low temperature, pasteurizing, fermentation, SGS, HPP, ultrasound processing, packaging, etc. where microbes face several smaller obstacles, which they need to overcome, making it difficult for them to thrive (Leistner, 2000), as illustrated in Figure 2.1.

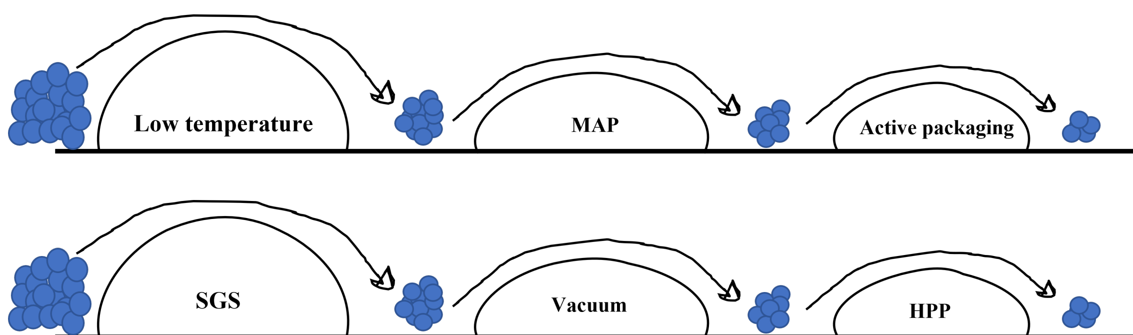


Figure 2.1: The term hurdle technology is used when several less invasive technologies are used to preserve a food product and prolong its shelf life. The figure illustrates two different suggestions for combinations, where the microorganisms (blue cluster) decreases for each hurdle.

2.2 Food packaging

The food packaging's main function is to serve as an gas-tight barrier, keeping the food safe from its surroundings. It prevents contamination and maintains flavor and color. Additionally, the packaging can be used as a tool by manufacturers to communicate vital information to the customers, including ingredients and nutritional values. By adopting innovative packaging techniques, such as intelligent packaging methods, customers can also determine the product's quality status. Manufacturers can communicate their values by opting for eco-friendly packaging materials and obtaining relevant certifications to showcase their commitment to sustainability. This can enhance the manufacturer's credibility and make it easier for customers to make an informed decision while choosing their product.

The packaging of fish products is crucial as fish are highly perishable and tend to spoil quickly in ambient air. Therefore, the materials used for packaging must possess excellent water and gas barrier properties along with a secure sealing mechanism (Boziaris, 2014). One way to achieve this is by layering multiple packaging materials with varying properties. Commonly used materials for this purpose include polymer structures like polypropylene (PP), polyethylene (PE), polyamide (PA), and polyethylene terephthalate (PET). However, such multi-layered plastics are primarily made from fossil-based polymers and are not recyclable nor biodegradable. Thus, the interest in more sustainable packaging is increasing. Several new packages have been introduced to the food industry, like those made from biodegradable polymers (Paul et al., 2022). These natural materials are derived from renewable sources such as plants, animals, and microorganisms. Polysaccharides which is frequently used in biodegradable packaging, include alginate, carrageenan, chitosan, cellulose, pullulan, and starch derivatives (Paul et al., 2022).

To ensure food quality, the packaging needs to have the optimal permeability characteristics for the particular food product. Permeability refers to the measurement of how much gas or vapor (the permeate) can pass through a material that resists it (Siracusa, 2012). The diffusion model (Figure 2.2) explains how gas passes through a material made of polymers.

The model includes Henry's law for adsorption and desorption, as well as Fick's law (Equation 2.1) for one-dimensional diffusion through a polymer membrane at stationary conditions,

$$J = -D\frac{\Delta c}{l} \tag{2.1}$$

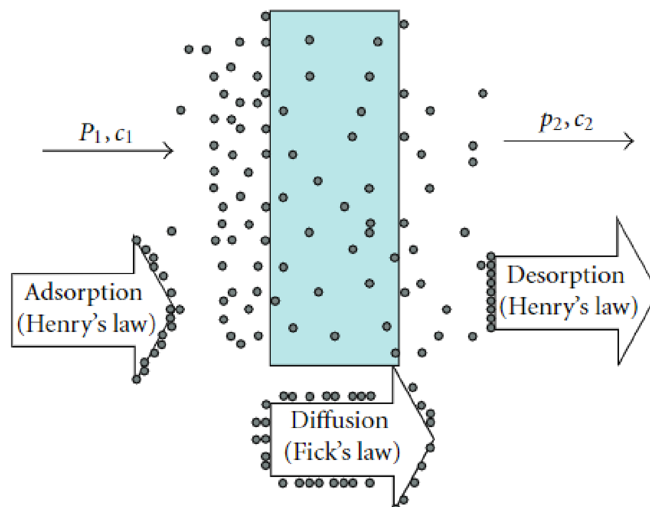


Figure 2.2: The permeation of gas or vapor through a polymer membrane with the thickness, l, can be explained by Henry's law and Fick's law when the permeate pressure and concentration are $p_1 > p_2$ and $c_1 > c_2$ respectively. Credit: Siracusa, 2012.

where J is the permeate flux, D is the diffusion coeffsient, and Δc is the concentration difference across the membrane thickness (l) when $c_1 > c_2$, and $p_1 > p_2$. The diffusion coefficient represents the rate at which the permeate diffuses through the membrane and is influenced by various factors, such as the size, shape, and polarity of the molecule, as well as the crystallinity, cross-linking, and motion of the polymer matrix (Siracusa, 2012).

The packaging's ability to prevent the permeation of water vapor, oxygen, and carbon dioxide can be described by the oxygen transmission rate (OTR), the water vapor transmission rate (WVTR), and the carbon dioxide transmission rate (CO2TR) (Siracusa et al., 2008; Siracusa, 2012). The transmission rates (TR) [cc/(m2s)] of substance x (O2, CO2, or water vapor) can be calculated based on Fick's law, by equation 2.2,

$$xTR = xPC\frac{\Delta p}{l} \tag{2.2}$$

where the value xPC [kgm$/($m^{2}sPa$)$] represents the permeability coefficient of substance x. xPC determines the amount of oxygen, carbon dioxide, or water vapor that passes through the packaging per unit area and time. The thickness of the polymer film is denoted by l [m], and the difference in partial pressure across the polymer film is represented by $\Delta p = p_1 - p_2$ [Pa], where p_1 is the partial pressure of substance x on the inside of the packaging and p_2 is equal to zero on the detector side (Siracusa, 2012).

Bio-plastic films may have some limitations, such as poor heat seal ability, thermal instability, high water vapor permeability, brittleness, and low melt strength. However, over the years, the production of bioplastic films has evolved, using nanotechnology to incorporating renewable sources such as starch, cellulose, plasticizer, and other additives (Othman et al., 2020; Pandey et al., 2015). This thesis studies the properties of compostable packaging made of cellulose film laminated to BioPBS (Biobased PolyBytulene Succinate) by comparing it to laminated multi-material (PA/PE).

2.2.1 Cellulose film laminated to BioPBS

Poly(butylene succinate) (PBS) consists of succinic acid and 1,4-butanediol monomers. It is a biodegradable polymer frequently used in food packaging due to its good mechanical strength and wide availability on an industrial scale (Chen and Yan, 2020; Nilsen-Nygaard et al., 2021). It is also relatively resistant to degradation when exposed to heat, light, and chemicals, making them suitable for food packaging applications (Nilsen-Nygaard et al., 2021). The monomers used in PBS production are traditionally extracted from fossil feedstock. However, recent advancements have enabled their production from renewable biomass like starch, glucose, and xylose on an industrial scale (Chen and Yan, 2020; Nilsen-Nygaard et al., 2021). PBS is a semicrystalline polymer with high crystallization ability and a melting temperature higher than most poly(alkylene dicarboxylate)s (Nilsen-Nygaard et al., 2021). Blending PBS with other biodegradable polyesters can yield even better properties and characteristics (Cherykhunthod et al., 2015).

Cellulose is long polymer chains containing exclusively of 1,4-linked β-D-glucopyranose units linked by hydrogen bonds. It is a natural occurred fiber which can be biosynthesized by several living organisms such as plants, sea animals, bacteria, and fungi (Othman et al., 2020). Because of its high degree of crystallinity and poor water solubility, it can improve the moisture barrier of polymer films by lowering its permeability (Pandey et al., 2015).

Cellulose film laminated to BioPBS (technical data sheet in Appendix A) is a compostable duplex laminate package with a thickness of 44.1-53.9 µm, and its target OTR and WVTR are < 1 cc/(m^{2}24 h) and < 14g/(m^{2}24 h), respectively. The sealing temperature is targeted at 100 °C, however it ranges from 80 °C to 140 °C.

2.2.2 Multi-layered material PA/PE

The food industry commonly uses multi-layered PA/PE packaging for packaging purposes for many years. PA has good gas barrier properties. However, it depends on the relative humidity (Boziaris, 2014). Thus, combining it with PE, which has good water barrier properties, will be beneficial.

The multi-layered non-recyclable vacuum bags used for this thesis is made of a transparent co-extrusion PA/PE film (technical data sheet in Appendix B). Its thickness is 80 µm ±5%, and its target OTR and WVTR are $\sim$ 52 cc/(m^{2}24 h) and 2.3g/(m^{2}24 h), respectively. The sealing temperature can range from 140 °C to 160 °C.

2.3 Fish quality

When it comes to evaluating fish, visual cues are key indicators of quality. A healthy fish should have bright, iridescent skin with no signs of discoloration. The cornea of its eye should be transparent, with a bright black pupil. Additionally, the gills should be a vibrant shade of red (Huss, 1995). When a fish is fully filleted, these parts are no longer present. Therefore, it is necessary to examine other parameters such as the color and texture of the flesh, as well as its smell. The flesh should have a firm and elastic consistency, with a translucent bluish color (white fish), while emitting a fresh seaweed scent. Additional factors, including microbial growth, changes in pH levels, water-holding properties, and nutritional content, may also be beneficial to investigate.

2.3.1 Fish spoilage

Fresh fish is highly perishable and spoils easily. Thus, handling it with proper caution is crucial to extend its shelf life. After death, the fish spoilage mechanisms can be divided into two groups: autolytical degradation and microbial degradation. Autolytical degradation is the first step where the fish still holds active enzymes. Thus, the term ”self-digestion” has been commonly used. The enzymes initiate several mechanisms by which cells and tissue deteriorate (Huss, 1995). After the fish is newly caught, the fish muscle is considered sterile. However, when the fish is processed, the microbes on the fish’s outer skin layer can contaminate the muscle, and microbial degradation begins. The microbes on the fish’s skin adapt to the temperature of the surrounding water. Thus, storing the fish at a low temperature immediately after

capture is crucial to inhibit the growth of unwanted microbes. Only a few microorganisms in the fish are responsible for the spoilage. These microorganisms are known as specific spoilage organisms (SSO) and vary among and within species, depending on how the fish has been handled and processed. An overview of the dominant microflora of Atlantic cod and its corresponding SSO can be found in Huss (1995). Cod stored on ice in a vacuum or aerobic conditions has *Aeromonas spp.* and *Shewanella putrefaciens* as SSO, and cod stored in MAP has *Photobacterium phosphoreum* as SSO (Sivertsvik, Jeksrud et al., 2002). The hydrogen sulphide producing bacterias, *Aeromonas spp.* and *S. putrefaciens*, contribute to the off-flavor and odor of the fish. Aerobic count on iron agar can determine the spoilage of these bacteria (NMKL method Nr. 184, 2006). Under anaerobic conditions, hydrogen sulfide production is a step in the reduction mechanism of trimethylamine oxide (TMAO) to trimethylamine (TMA). The rancid smell and flavor that occur when fish deteriorate are due to this lipid oxidation. *P. phosphoreum* is a psychrotolerant microorganism that is resistant to CO_2, and has been identified as a spoilage microorganism for vacuum packaged and modified atmosphere packaged fish (Dalgaard and Huss, 1993). Thus, *P. phosphoreum* might survive the SGS treatment. Aerobic plate counts on Long & Hammer (L&H) agar (NMKL method Nr. 184, 2006) can determine spoilage due to *P. phosphoreum*. When the total aerobic plate count (TAPC) or the total psycotrophic count (TPC) reaches 10^7 cfu g^{-1}, the fish are considered spoiled (Huss, 1995).

2.3.2 Surface color

The surface color is an important quality parameter as it can indicate the freshness of the fish. The flesh of a fresh cod should be bluish, translucent, smooth, and shining, and there should be no changes in the original color (Huss, 1995).

The Hunter system (CIELAB system) can be used to describe the surface color of the fish muscle (Coultate, 2016). The system is based on a three-coordinate system (Figure 2.3). L^*, the vertical coordinate, refers to the lightness and varies from black ($L^* = 0$) to white ($L^* = 100$). The two horizontal coordinates, a^* and b^*, refer to the complimentary colors red and green, and yellow and blue, respectively, where -a^* to +a^* refers from green to red, and -b^* to +b^* refers from blue to yellow.

The chroma value,C^*, reflects the purity of color and is related to the different color variations between the coordinates. A high chroma value refers to a color with minimal added black,

white, or gray. The hue angle, h°, relates to the relative amount of red ($h° = 0°$), yellow ($h° = 90°$), green ($h° = 180°$) and blue ($h° = 270°$). The chroma and hue value is defined by equations 2.3 and 2.4. Chroma reflects the distance from the center of the coordinate system, while the hue angle is the angle in the vertical plane. Chroma (C^*) and hue (h°) are defined by a three-dimensional coordinate system based on polar coordinates, where x = rcos(ϑ), y = rsin(ϑ), and the z-axis is vertical. The chroma value, C^*, refers to the radius r, the hue value refers to the angle ϑ, and lightness, L^*, is the vertical axis z.

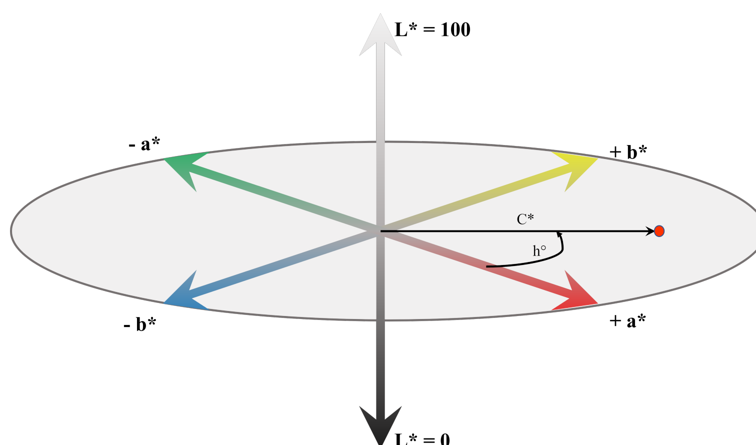


Figure 2.3: The CIELAB color system defines the different color variations. L^*, the vertical coordinate, refers to the lightness and varies from black ($L^* = 0$) to white ($L^* = 100$). The two horizontal coordinates, a^* and b^*, refer to the complimentary colors red and green, and yellow and blue, respectively, where $-a^*$ to $+a^*$ refers from green to red, and $-b^*$ to $+b^*$ refers from blue to yellow. Chroma, C^*, refers to how much black, white, and gray are added to the color, and the hue angle, h°, relates to the relative amount of red ($h° = 0°$), yellow ($h° = 90°$), green ($h° = 180°$) and blue ($h° = 270°$).

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{2.3}$$

$$h° = \arctan(\frac{b^*}{a^*}) \tag{2.4}$$

2.3.3 Texture

Texture in the context of food is mainly related to the mouthfeel. It is a critical quality parameter, as consumers often have a predetermined idea of a food product's texture. If the texture deviates far from the origin, the customer will not be motivated to repurchase. The best way to judge and

determine the texture is, according to Lewis (1990), by a sensory panel, as the texture is more of a psychological or sensory parameter rather than a physical parameter. The texture of the fresh fish muscle should be firm but yet elastic with a smooth surface (Huss, 1995). However, a fish product such as clip-fish, which has been pre-processed, would be more acceptable to deviate from the texture of fresh fish in contrast to a non-processed cod.

In recent years, it has become more common to evaluate the texture based on physical measurements. The industry has developed new technology and instruments, such as TA.XTplus texture analyzer, to measure and evaluate the texture. These instruments measure the texture based on physical parameters, such as how much force is needed to deform the product (force) or how much force is needed to press down a pre-decided percentage of the height. A significant amount of data can be collected and compared using this type of instrument (Lewis, 1990).

2.4 Cod muscle

Fish exhibit a wide range of chemical compositions, which vary according to their species, but also at an individual level depending on age, sex, environment, and season (Huss, 1995). Fish muscle, in general, has a low concentration of carbohydrates ($< 0.5\%$), and cod is a lean fish with a lipid content of 0.1–1% with little seasonal variation (Huss, 1995; Gordon, 2003). Thus, the chemical composition of cod muscle mainly consists of water and protein. Fresh cod generally holds 78-83% water (Huss, 1995). According to Bisenius et al. (2019) the main composition of the Atlantic cod (*Gadus morhua*) caught in February 2016 were $79.4 \pm 0.9\%$ water, and 19.6 ± 0.9 % protein, with a water/protein ratio of 4.1 ± 0.2.

2.4.1 Protein and protein denaturation

Fish proteins can be classified into three categories - structural protein, sarcoplasmic protein, and connective tissue protein (Huss, 1995). The most abundant group of proteins is the structural proteins, which account for 70-80% of the total protein content, and are located in the myotomes of the fish muscle (Figure 2.4). This group comprises actin, myosin, tropormyosin, and actomyosin. The sarcoplasmic proteins make up 25-30% of the total protein content and consist of myoalbumin, globulin, and enzymes. The connective tissue protein is the smallest group, representing only 3% of the total protein content, and includes collagen.

When proteins are exposed to thermal treatments, high salt concentrations, or high pressure,

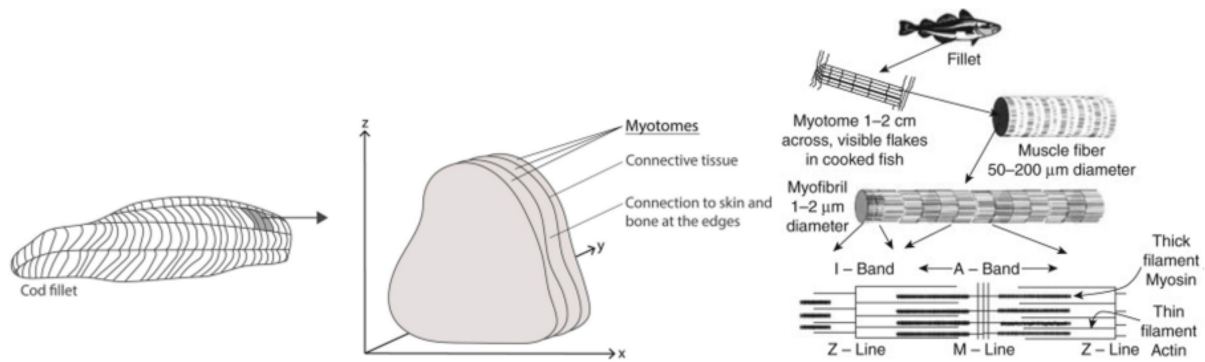


Figure 2.4: The thin stripes in the fish muscle are the connective tissue, which hold the myotomes together. The muscle fibers placed in the myotomes, which in turn contain the structural proteins myosin and actin. Credit: Blikra et al., 2020; Phillips and Williams, 2011.

protein denaturation occurs (Huss, 1995; Di Luccia et al., 2005; Thorarinsdottir et al., 2001; Martínez et al., 2017). It is a modification of the secondary, tertiary, or quaternary conformation of the protein, where the protein unfolds. The peptide bonds in the primary structure do not rapture, resulting in a totally or partially unfolded polypeptide structure (Figure 2.5) (Messens et al., 1997; Huss, 1995). Protein denaturation can be detected using differential scanning calorimetry (DSC), which is very helpful when studying proteins thermodynamic stability (Thorarinsdottir et al., 2001). DSC works by measuring the energy transfer during physical or chemical changes in a sample and recording the temperature at which the transfer occurs. Table 2.1 hold an overview of the different denaturation temperatures of myosin, actin, and sarcoplasmic proteins of different fishery products based on previous studies.

A study by Di Luccia et al. (2005) measured the effects of the hydration process on water-soluble proteins of stockfish and salted cod. They concluded that the salting involves the diffusion of salt into fish muscle tissue, leading to the denaturation of sarcoplasmic and myofibrillar proteins as well as possible solubilization of myofibrillar protein and heavy myosin chain fragmentation. They also believed that the high salt concentration initially caused protein loss due to the osmotic effect of the salt. Martínez et al. (2017) investigated how HPP at 100, 300 and 600 MPa (10 °C/5min) affected the protein fraction of blue crab (*Callinectes sapidus*). The study concluded that by using HPP, the yield of extracted crab meat increased compared to other treatments, due to the protein denaturation. As the pressure level increased, the denaturation temperature and the enthalpy of myosin significantly decreased. These findings indicate that the level of pressure affects the amount of denatured protein.

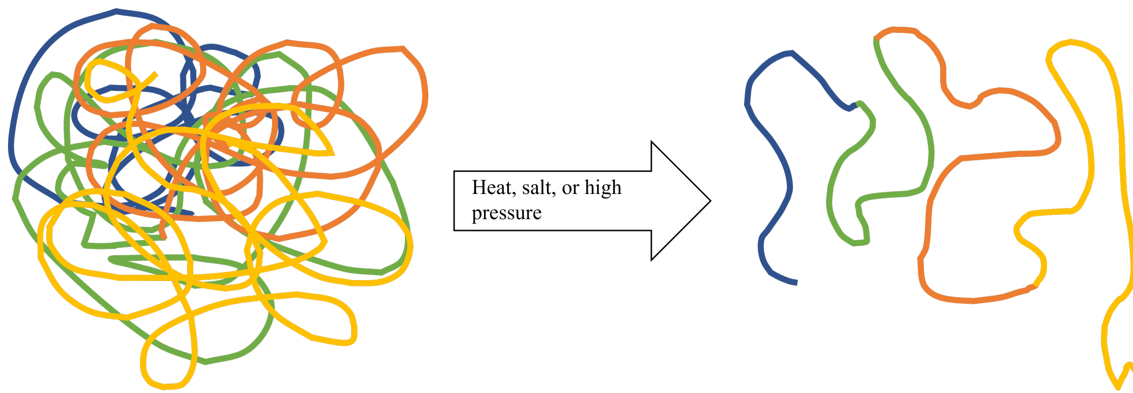


Figure 2.5: When protein is exposed to thermal treatments, high salt concentrations, or high pressure, it will denaturate. This leads to a modification of the secondary, tertiary, or quaternary conformation of the protein, where the protein unfolds.

Table 2.1: Denaturation temperatures (T_{max}) for myosin, actin, and sarcoplasmic proteins in cod muscle found in literature.

Product	Protein	T_{max} [°C]	Reference
Atlantic Cod, fresh	Myosin	~ 43.5	*Thorarinsdottir et al. (2001)*
		44.1 ± 0.2	*Skipnes et al. (2007)*
	Actin	~ 73.6	
		76.1 ± 0.7	
	Sarcoplasmic proteins	~ 59.3	
		57.3 ± 0.1	
Atlantic cod, stored at -30 °C, and -10 °C for 120 days	Myosin	~ 36.03	*Badii and Howell (2002)*
		~ 35.31	
	Actin	~ 65.11	
		~ 66.41	

Numerous studies have proven that there are correlations between increasing drip loss (DL) and poorer texture (Campus et al., 2010; Cheng et al., 2014; Kristoffersen et al., 2007; Christensen et al., 2017). However, protein denaturation is considered to be the main reason for both water loss and textural changes in fish (Skipnes et al., 2007; Alves de Oliveira et al., 2017).

2.4.2 Water content and drip loss

The water in the fish muscle can be classified as bound water, immobilized water, or free water based on its mobility. Free water can freely migrate through the muscle and is weakly held by capillary forces. Immobilized water is entrapped in the capillaries and cells. However, it

flows freely and will escape as DL by damage or cutting. The free water and the immobilized water describe 95% of the total water in the fish muscle (Boziaris, 2014; Aursand et al., 2008). The bounded water has more structural bindings, and can not act as a solvent (Vaclavik et al., 2021). Bounded water is a term closely linked to water holding capacity (WHC), which refers to the maximum amount of bounded water a product can hold (Coultate, 2016). DL is defined as the protein-holding liquid that seeps out of the muscle when it is processed or stored. DL is calculated as the percentage weight loss of a product and is an important quality parameter (equation 3.1).

2.4.3 Free amino acid

Proteins are polymers of amino acids. There are 20 distinct amino acids that the body needs to build protein. However, the human body is only capable of synthesizing half of them (Nelson and Cox, 2017). Therefore, maintaining a balanced and nutritious diet is crucial to ensure that we obtain all the necessary amino acids.

Free amino acids (FAA) are not bounded to proteins, they are derived from the protein by proteolysis (Boziaris, 2014). The FAA is located in the free water of the fish muscle. Thus, an increase in DL leads to a decrease in FAA. Several studies have related FAA, such as alanine, glutamic acid, and glycine, to the distinct flavor of fisheries (Kendler et al., 2023; Hayashi et al., 1981; Li et al., 2009). A study conducted by Rodríguez-González et al. (2020) on the manufacturing process of Galician Chorizo Sausage revealed that certain FAA contributes to taste and odor compounds when degraded. The study also grouped FAA based on their flavor profile. For instance, some FAA like alanine, glycine, threonine, serine, and proline are sweet while leucine, valine, isoleucine, methionine, and phenylalanine have a bitter taste. FAA, such as glutamate, aspartate, and histidine, are acidic, while aspartate, tyrosine, and lysine give the sausage an aged flavor. As protein breaks down during storage, FAA increases. During bacterial spoilage, amino acids break down into malodorous compounds such as putrescine, cadaverine, and hydrogen sulfide, as well as ammonia. These end products are typically associated with fish putrefaction. As a result, microbial spoilage causes significant deteriorative changes in fish (Antoine et al., 2001; Boziaris, 2014). Thus, an increase in certain FAA contributes to off-flavor and taste during storage.

2.5 Clip-fish

Clip-fish is a Norwegian delicacy traditionally made from cod. It is salted and dried, historically outside on cliffs. However, nowadays, the drying process is performed indoors, where it is easier to control the drying conditions. After capturing the fish, it undergoes gutting and removal of the backbone to enable it to fold into its characteristic V-shape (Norwegian Seafood Council, 2022), as illustrated in Figure 2.6. This is followed by salting, which dissolves the salt in the water extracted from the muscle, creating a brine. The brine is then rinsed off, and the fish is salted again. It is left to dry for several weeks, after which the remaining water is pressed out. The fish is then salted for the third time and left to dry for the final period. When the water content is lower than 48%, the fish is classified as clip-fish (Norwegian Seafood Council, 2022).

Food with low water content has a long shelf life, as a low water activity will inhibit the growth of unwanted microorganisms (Coultate, 2016). Thus, the clip-fish in its dried state will have a long and stable shelf life. However, in the process of utilizing clip-fish for food production, it is necessary to rehydrate the fish due to its high salt content (Matvaretabellen, 2022a; Rode and Rotabakk, 2021). This increases the water activity, which accelerates the growth of microorganisms. Thus, at normal conditions, the fish needs to be consumed quickly after the rehydration. The rehydration will also lower the salt content, which is necessary for consuming it. The rehydration aims to reduce the salt content to an edible level, 2-3% (Nofima, 2022).

Figure 2.6: Clipfish as a finished product in its caracteristic V-shape.
Credit: Dybvik AS (Ålesund, Norway)

2.5.1 Effects of drying fish

The main effect of drying fish is the difference in the chemical composition, which according to Matvaretabellen (2022a) and Matvaretabellen (2022b) are 35% water and 64.3 % protein, resulting in a water/protein ratio of 0.54 for clip-fish, and 82% water and 17.4 % protein, resulting in a water/protein ratio of 4.7 for fresh Atlantic cod (the general composition of Atlantic cod is further explained in section 2.4).

The drying process has also a significant effect on the end product's qualities, particularly in regard to protein denaturation. However, protein nitrogen remains unaffected , resulting in alterations to the amino acid composition, protein solubility, and protein digestibility (Boziaris, 2014).

2.6 Mild processing technologies

Mild processing technologies refer to methods that can prolong the shelf life of products by partially or completely preventing spoilage and pathogenic microorganisms and/or enzymes, while minimizing any negative impact on the product's sensory properties, nutritional content, and characteristics (Abel, 2021). Superchilling, sous vide, HPP, SGS, curing and ultraviolet and pulsed light are all examples of mild processing technologies. The processing technologies used in this study are SGS and HPP. Both have been proven to prolong the microbiological shelf life. Research has shown that the parameters for HPP are determined by the type of food product, as the quality can be affected. By combining HPP with other gentle preservation techniques (known as hurdle technology, section 2.1), such as SGS, a reduction in pressure may be possible while maintaining satisfactory quality parameters. Also, clip-fish is already partially processed and does not have the fresh appearance associated with fresh cod. Thus, the sensorial properties of clip-fish may not be as affected as fresh cod.

2.6.1 High-pressure processing

HPP, also known as high hydrostatic pressure, is a technology that uses isostatic pressure to inhibit the growth of unwanted microorganisms in food products (Abel, 2021). This innovative technique utilizes isostatic pressure ranging from 100 MPa to 1000 MPa, which is transmitted through water pumped into a vessel where the food product is placed. The temperature of the water can be set to desired temperature, usually between 5 and 25 °C, and the holding time

usually varies between one and five minutes. It is an energy-efficient and effective preservation method for the fish industry, as it reduces production time and uses less energy than traditional thermal methods (Alba et al., 2019; Renaud et al., 2022). The method is widely used to preserve a range of food products. In the nineties, the first HPP-treated jam was introduced in Japan, followed by jellies and shellfish. In the United States, oysters and guacamole were first treated, and now several countries use the technology to preserve fruit juices, meal kits, poultry products, and sliced meat (Considine et al., 2008).

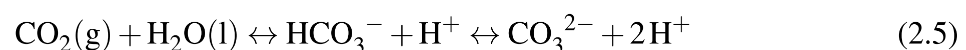
Figure 2.7: An illustration of an HPP system. The products are loaded into blue baskets which are then pushed into the vessel (holding chamber). Once inside, the vessel is sealed and water at a specific temperature is pumped in until the desired pressure is achieved. The pressure is maintained for a certain duration of time before it is released, allowing the product to emerge Credit: Hiperbaric (*Hiperbaric 55* n.d.)

According to research (Lakshmanan et al., 2007), the final quality of a food product can be affected in either a positive or negative way by HPP. This impact is determined by factors such as pressure level, temperature, holding time, and the specific product being processed. One of the key benefits of this process is prolonging the shelf life of food products by either reducing or inactivating microorganisms, thus prolonging their lag phase (Abel, 2021; Rode and Hovda, 2016). Additionally, this process also helps to maintain the fresh qualities of the food products (Campus et al., 2010). Numerous studies have demonstrated that various products, including sea bream (Campus et al., 2010), Atlantic salmon (Zhu et al., 2004), Atlantic cod and mackerel (Rode and Hovda, 2016), and different types of shellfish (Murchie et al., 2005), have a specific pressure threshold that can decrease microbial growth without negatively impact their texture, color, or WHC. The impact of HPP on frozen and re-thawed sliced cod and salmon was investigated by Arnaud et al. (2018). The study concluded that in order to maintain the microbial quality of the fish for two weeks, HPP needs to be performed at a pressure of 450 MPa for 5

minutes at 20 °C. However, when the pressure reached beyond 300 MPa, the texture and color of the fish were affected, giving it a processed appearance instead of a fresh. In a study on gilthead sea bream (*Sparus aurata* L.) by Campus et al. (2010), it was found that the WHC of the muscle decreased as pressure increased. In addition, Zhu et al. (2004) noted a significant change in the color of salmon fillets thawed during HPP above 150 MPa at 20 °C, with a thawing time of 18.17 ± 1.4 minutes. The salmon fillets became lighter in color with increasing pressure, and the texture was significantly modified with HPP at 200 MPa, where the salmon fillets reached their peak toughness. When Rode and Rotabakk (2021) treated rehydrated clip-fish with HPP at 400, 500, and 600 MPa, they concluded that the HPP could prolong the rehydrated clip-fish shelflife. However, the DL increased during storage, and by storage day 15, the clip-fish treated with 600 MPa had a significantly higher DL than the clip-fish treated with 400, and 500 MPa. After undergoing HPP treatment at 600 MPa, the clip-fish remained microbial-safe even after 49 days.

2.6.2 Soluble gas stabilization

SGS is a more recent technology compared to HPP. The concept was initially introduced by Sivertsvik (2000), and this method involves dissolving CO_2 into the food product. To achieve this, a 100 % CO_2 atmosphere is created inside a heat-sealed bag, and the product is kept in a refrigerated space at 4 °C for 12-18 h. According to research (Sivertsvik, Rosnes et al., 2004; Rotabakk and Sivertsvik, 2012), when pressure is increased at low temperatures, the solubility of CO_2 also increases. As a result, CO_2 will partially dissolve into the water- and oil phases of the product according to equation 2.5 (Mendes, Pestana et al., 2008; Mendes and Gonçalves, 2008).



$$CO_2(g) + H_2O(l) \leftrightarrow HCO_3{}^- + H^+ \leftrightarrow CO_3{}^{2-} + 2H^+ \qquad (2.5)$$

After a while, the water phase will contain dissolved CO_2 due to the equilibrium that occurs (Devlieghere et al., 1998). CO_2 has antimicrobial and antifungal properties which inhibit microbial growth and ultimately prolong the shelf life of the product. The bacteriostatic effect is said to be proportional to the CO_2 concentration in the water- and oil phase of the product (Devlieghere et al., 1998; Mendes and Gonçalves, 2008). SGS is considered a pre-step process and can be used as an alternative or addition to MAP to enhance the shelf life of the product

(Esmaeilian et al., 2021; Rotabakk and Sivertsvik, 2012).

Several research studies have explored the use of SGS as a novel pre-treatment for HPP, MAP, or other thermal treatments (Al-Nehlawi et al., 2014; Dang et al., 2021; Mendes and Gonçalves, 2008; Abel et al., 2019). According to Al-Nehlawi et al. (2014), the effectiveness of SGS can be improved by HPP as it has the ability to break down cell membranes in poultry sausages. This makes it easier for CO_2 to dissolve and results in a more potent impact on microorganisms. Additionally, Dang et al. (2021) found that while HPP has the main effect in prolonging the microbe's lag phase in precooked chicken breast packed in vacuum, SGS also contributes positively. However, they observed that the precooked chicken breasts' color darkened immediately after SGS treatment, but after two weeks of storage, the color returned to normal. Thus, by combining these technologies a lower pressure level in HPP can be used to achieve the desired inactivation of microbial growth.

2.6.3 The effect of HPP on food packaging

Different processing treatments can induce changes in the material and properties of food packaging. Several studies have investigated the effect HPP has on packaging material (Zhao and Tang, 2017; Le-Bail et al., 2006; Lambert et al., 2000). Due to the intense deformation of the packaging during HPP, the material of the packaging need to be flexible and heat sealable (Guillard et al., 2010).

Zhao and Tang (2017) tested the mechanical- and barrier properties of 80 µm thick PA/PE and 120 µm thick PET/PE packed with olive oil, which was subjected to hydrostatic pressure ranging from 200 MPa to 500 MPa at 25 °C. Due to decreased packaging volume during HPP, followed by varying recovery degrees, their study concluded that the barrier properties significantly changed, with a declining permeability coefficient for both PA/PE and PET/PE. Thus, the HPP treatment enhanced the packaging permeability properties. Le-Bail et al. (2006) investigated changes in mechanical properties and the water vapor permeability due to HPP (at 200, 400, and 600 MPa) in different types of non-recyclable packaging materials (PA-PE, PET/BOA/PE, PET/PVDC/PE, PA/SY LDPE, and EVA/PE). This study concluded that HPP did not significantly affect the packaging concerning the water vapor permeability or the mechanical strength of the packaging. Lambert et al. (2000) tested the integrity and the barrier and flexibility of PA/PE food packaging with varying thickness, permeability, and stress when exposed to HPP

at 200, 350, and 500 MPa for 30 min. All, but one, of the packages tested in the study were not significantly affected by the HPP treatments considering tensile strength, heat-seal strength, or delaminations. The one that did show a significant effect was due to demolition. None of the packagings showed any significant changes considering OTR or WVTR.

There is not much research available regarding the effect of HPP on biodegradable food packaging. A review paper of Guillard et al. (2010) gives an overview of different food processing methods' effects on the packaging, where they also concluded that they could not find any literature dealing with HPP and biodegradable packaging. However, they referred to several studies dealing with the effect of pressure on agro-polymers' gels to give an idea of what might happen to biodegradable polymers. These gels are made of hydrated materials. Thus, it might not be comparable, and further investigation is needed.

3 Material and Methods

This experiment aimed to determine how SGS and HPP treatment influence bio-based food packages compared to the conventional multilayered PA/PE food package. Thus, the different variables were 100% CO_2 (SGS), HPP at 400 MPa or 600 MPa, and the different packaging materials (BioPBS and PA/PE). Table 3.1 presents the different treatment groups' respective parameters. A flow diagram of the treatment plan is presented in Figure 3.1. The shelf life study was performed in two replicates, each with three parallels for each treatment group. The study period was 54 days and took place at Nofima AS, Stavanger.

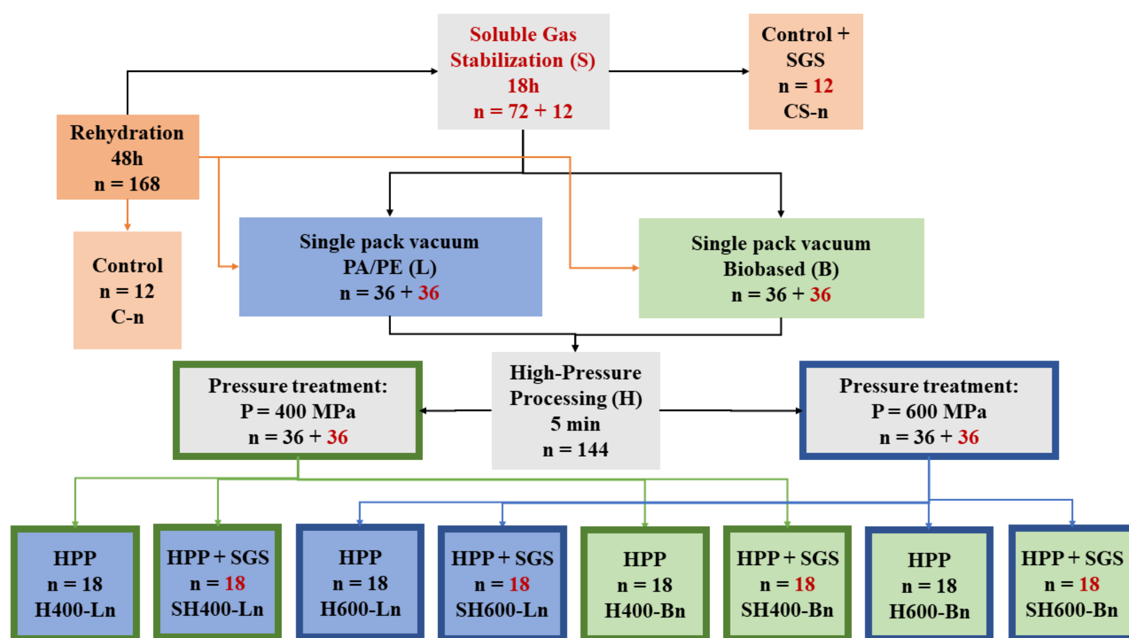


Figure 3.1: The flow diagram maps out the treatment plan for the clip-fish loins (n = the sample number). The red font color marks the samples treated with soluble gas stabilization (SGS). The different colors of the blocks represent the control groups (light orange), samples packed in PA/PE packaging (blue), and the samples packed in BioPBS packaging (green). The colors of the different frames represent the pressure level of the high-pressure processing (HPP) the groups have been treated with; 400 MPa (green), 600 MPa (blue)

.

3.1 Treatment of samples

In preparation for the experiment, pre-cut, skinned, and deboned clip-fish loins (Atlantic cod, n = 336) were ordered from Dybvik AS (Ålesund, Norway). Before rehydration, water was pre-cooled to 4 °C.

Table 3.1: The shelf life study had a total of ten treatment groups, where the C, S, and H are short for Control, SGS, and HPP, respectively. 400 and 600 refer to the different pressure levels of HPP, and B and L refer to the packaging, biobased (BioPBS), or laminate (PA/PE).

Group	Label	Parameter	Total n
1	C	Control - 0.1 MPa without SGS	12
2	CS	Control - 0.1 MPa with SGS	12
3	H400-L	HPP 400 MPa, 5 min (PA/PE bags)	18
4	SH400-L	SGS + HPP 400 MPa, 5min (PA/PE bags)	18
5	H400-B	HPP 400 MPa, 5 min (Bio-based bags)	18
6	SH400-B	SGS + HPP 400 MPa, 5min (Bio-based bags)	18
7	H600-L	HPP 600 MPa, 5 min (PA/PE bags)	18
8	SH600-L	SGS + HPP 600 MPa, 5min (PA/PE bags)	18
9	H600-B	HPP 600 MPa, 5 min (Bio-based bags)	18
10	SH600-B	SGS + HPP 600 MPa, 5min (Bio-based bags)	18
Total			168

3.1.1 Rehydration

The clip fish loins (25 kg) were spread on steel grates and placed in a 1000 L PE container, before 150 L of the pre-cooled water were added (1:6) using an electric pump (Figure 3.2). The container was held in chilled storage at 4 °C throughout the rehydration period. The water was drained and replaced after 6 h, 12 h, 24 h, and 48 h. At time 0 h, 8 random clip-fish loins were collected from the PE container, weighed, and placed back (Figure 3.2). These were also weighed each time the water was drained for 10 min and replaced. At the end of the rehydration period, the clip-fish were drained for 10 min before further treatment and vacuum packaging.

3.1.2 Soluble gas stabilization

After the rehydration, half of the clip-fish loins (n=84) were randomly picked and treated with SGS for 12 h. The rest were placed in steel grates packaged in air first before vacuum packaging all of them after 12 h. The clip-fish loins were randomly distributed on 5 steel grates placed in PA/PE 80 m bags (450 mm × 700 mm, NorEngros, Norway). An atmosphere of CO_2 was fabricated inside the bags using a Webomatic vacuum chamber connected to 100% CO_2 gas. To control the gas composition of the headspace gas, an empty tray was placed inside a bag and filled with 100% CO_2. Then, using a PBI Dansensor CheckMate 9900 Headspace Gas Ana-

Figure 3.2: Clip-fish loins spread out in the polyethylene container. The loins in the blue basket were weighed and registered after 6, 12, 24, and 48 hours.

lyzer (Nordic Supply System, Norway), the gas composition (CO_2 and O_2) was measured and registered (three parallels). The bags were then placed in a cold room at 4 °C for 12 h. Thereafter, the headspace gas was measured before individually vacuum packing the SGS-treated loins (section 3.1.3).

3.1.3 Packaging

After rehydration, the clip-fish was drained of excess water for 10 minutes. The clip-fish loins were single packaged with 99% vacuum using a Webomatic vacuum chamber (Werner Bonk, Bochum, Germany). The control groups (C, CS), and those packaged in the multi-layered bags (groups H400-L, SH400-L, H600-L, and SH600-L), were vacuumed and heat-sealed in 20 m PA/70 m PE bag (180 × 140 mm, Lietpak, Vilnius, Lithuania), while those packaged in bio-based packaging (groups H400-B, SH400-B, H600-B, and SH600-B) were vacuumed and heat-sealed in cellulose film laminated to BioPBS bags (250 x 160 mm, Grounded Packaging, Sydney, Australia). All the single packages were marked, weighed, and registered before the HPP treatment (Table 3.1).

3.1.4 High-pressure processing

The clipfish were treated at 400 and 600 MPa at 8 – 9°C using a high hydrostatic pressure machine QFP 2L-700 (Avure echnologies Inc., Columbus, USA) for 5 minutes (not including the come-up time). QFP 2L-700 is a small-scale HPP model for research and development.

It consists of a control system where the set-points pressure, temperature, and dwell time can be regulated and a pressure vessel made of stainless steel. The single-packaged clip-fish were packaged and sealed in bags PA/PE 20/70 my bag (Lietpak, Vilnius, Lithuania), (four single packages in each bag) before they were placed in the work basket, which was placed in the pressure vessel. The pressure cycle was initiated when the vessel closed and the pressure chamber was filled with water. The come-up time was ~65 s and ~100 s for 400 and 600 MPa, respectively, whereas the pressure release was immediate. After the treatment, the samples were placed in buckets with ice water for rapid chilling before cold storage.

3.1.5 Storage

After the HPP treatment, all 168 samples of each replication were stored in a chilled room at 4 °C. The samples were divided into 10 groups marked according to Table 3.1. For each sampling day, 3 sample loins of each treatment group were chosen randomly for further quality measurements.

3.2 Quality measurements

In this thesis, the quality and shelf life of rehydrated clip-fish treated with SGS and/or HPP and packed in two different packagings were evaluated based on changes in microbial growth, DL, water content, surface color, texture, salt content, protein content, protein denaturation, and free amine content.

On each sampling day, the loins were immediately weighed after opening the packages to determine any DL (equation 3.1). Then the loins were sliced into three pieces as illustrated in Figure 3.3. Around 4-5 g and 10 g were sliced for water content and microbial analysis, respectively, the rest of the loins were used to perform color- and texture measurements. After the texture measurements were completed, the loins were cut into three pieces, vacuum packaged, and frozen at −80 °C for further analysis of protein denaturation, salt content, protein content, and free amines.

$$DL[\%] = \frac{\text{initial weight [g]} - \text{weight after processing [g]}}{\text{initial weight [g]}} \cdot 100\% \tag{3.1}$$

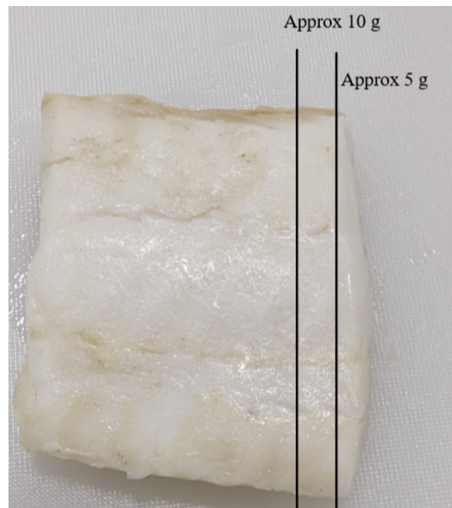


Figure 3.3: The clip-fish loins were divided into three pieces, 4-5 g for water content measurements, 10 g for microbiological analysis, and the rest for color- and texture measurements before freezing at -80 °C for further analysis of salt- and protein content, protein denaturation, and free amino acid content.

3.2.1 Water content

To evaluate the water content in the clip-fish, the small pieces of approx 5 g were weighed and registered in small aluminum dishes. Then, they were placed in a heating cabinet holding 105 °C for 18±6 h. The weight of the dried matter was then recorded, and water content was calculated using equation 3.2,

$$\%WC = \frac{w_0 - (w_{18} - w_d)}{w_0} \cdot 100\% \tag{3.2}$$

where w_0 is the weight of the sample before drying, w_d are the weight of the aluminum dish used and w_{18} are the weight of the sample after 18± 6 h drying at 105 °C.

3.2.2 Microbiology analysis

TAPC, total TPC, and hydrogen sulphide producing bacteria count (HSPB) were determined according to the NMKL method Nr. 184 (2006). The small bits of clip-fish muscle, 10 g, were diluted 1:9 separately with sterile peptone water in stomacher bags and homogenized for 2 min using a Stomacher 400 Laboratory Blender (Seward Medical, London, UK). After the homogenization, the bags were left on stands, so the bacterial culture was separated from the foam. Then the culture was poured into 50 mL centrifuge tubes. Appropriate dilution series were made in 2 mL sterile centrifuge tubes, according to Table C.1 depending on the sample

day. 1 mL from the dilution, was transferred to petri dishes before 10-12 mL sterile iron agar containing *L*-cysteine were poured over. 49.2 µL of the solution was plated mechanically on L&H agar using an EddyJet (IUL Instruments, Barcelona, Spain), according to Table C.1. The iron agar and L&H agars were incubated at 25 °C for 72 ± 6h and 15 ± 1 °C for 5-7 days to quantify for TAPC and TPC, respectively.

The black and total colonies from the iron agar quantify for HSPB and TAPS, respectively, while the total colonies from the L&H agar quantify for TPC. The pH of the homogenized samples was measured at room temperature from the centrifuge tubes using a pH meter (Mettler Toledo FiveEasy Plus with LE410 electrode).

3.2.3 Surface color

The surface color of the clip-fish loins was measured using a DigiEye full system (VeriVide Ltd., Leicester, UK), which consisted of a standardized light-box that fabricated daylight at 6400 K (Figure 3.4), and a Nikon D80 camera (35 mm lens, Nikon Corp., Japan). The software Digipix (version 2.8, VeriVide Ltd., Leicester, UK) was used to quantify for L^*, a^*, and b^* values, where L^* describes lightness, a^* and b^* represents redness and yellowness, respectively. The system was calibrated before each analysis. The hue and chroma values were calculated using equation 2.4 and 2.3, respectively.

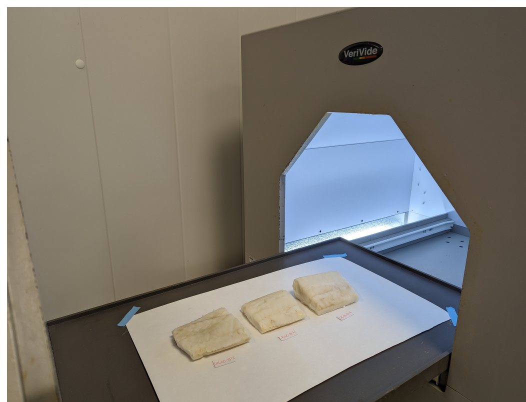


Figure 3.4: Clip-fish loins ready for color measurements by the DigiEye full system.

3.2.4 Texture analysis

The texture of the clip-fish was measured using a TA.XTplus texture analyzer, equipped with a 50 kg load cell and a cylinder probe with a diameter of 1/2" (12.7 mm), Type P/0.5 (Figure 3.5). The software, Exponent version 6.1.16.0 (Stable Micro Systems, Surrey, UK) was used to

collect the data. The loins were transferred straight from the ice onto the platform of the texture analyzer, and the probe was pressed down to 60% of the sample height at a rate of $2\,\mathrm{mm\,s^{-1}}$, which created punctures in the loins. The texture was measured in 2 replicates, and data for the fillet height [mm], the breaking force [N], and the firmness [N] (measured by force at 60% compression of the initial height) were recorded.

Figure 3.5: TA.XTplus texture analyzer setup.

3.2.5 Salt content

The salt content was measured in all the samples taken at day 0 by performing the Mohrs method with an Easy Cl automatic titrator (Mettler Toledo, Norway), which automatically calculates the percentage NaCl content in the fish based on the amount of sample according to Equation 3.3. where Eq is the volume [mL] of $AgNO_3$ consumed at equivalence point; $B = 0$, blank value; $T = 0.1\,\mathrm{mol\,L^{-1}}$, the concentration of titrant; $M = 58.44\,\mathrm{g\,mol^{-1}}$, molecular weight of NaCl; $F1 = 0.1$, conversion factor for % and W = sample weight [g].

$$\%\mathrm{NaCl} = \frac{(Eq - B)) \cdot T \cdot M \cdot F1}{W} \tag{3.3}$$

Frozen clip-fish samples were thawed and 0.5 g were cut from the middle of the loin, weighed and homogenized with 100 mL distilled water (55 °C) for 40 s at 13 500 rpm and 1 mL HNO_3

(1 M) were added before the titration began. The titration stopped at the equivalence point when AgCl was formed.

3.2.6 Protein denaturation

Any differences in protein denaturation between the treatment groups on day 0 were analyzed using the DSC analyzer system (Mettler Toledo, Norway) together with STAR software. The start and end temperatures were set to 2 °C and 110 °C respectively, with a heating rate of 5 °C min^{-1}. Frozen clip-fish were thawed and approx 50 mg clip-fish (taken from the middle of the loin) were weighed in small steal crucibles (ME-26929, Ø 7 mm, with pin). A reference with distilled water was used with the amount of water that was equivalent to the water content in the clip-fish (79.4 %). However, the groups treated with 400 MPa (section 4.11) gave uneven results. It was assumed that the clip-fish treated with 600 MPa would not give any clearer result. Thus, protein denaturation was only measured in the clip-fish treated with 400 MPa.

3.2.7 Protein content

The protein content of the clip-fish samples from days 0, 24, and 48 was determined using the Kjeldahl method according to Application Note No. 114/2013 *Nitorgen & protein determination in meat products* (Büchi, Switzerland). This method is divided into three steps:

1. Homogenization of deep-frozen samples with a Braun MultiQuick 5 Vario hand blender MQ5235BK.
2. Digesting samples using KjelDigester K-449 connected to Scrubber K-415 TripleScrub (Büchi, Switzerland)
3. Distillation and titration of the samples using KjelMaster K-375 with KjelSampler K-376 (Büchi, Switzerland)

The samples were transferred from the frozen storage and homogenized using a Braun MultiQuick 5 Vario hand blender. One analysis could hold 20 sample tubes. The first two tubes held blank samples (holding only a Watman B2 nitrogen-free paper), the third and the 20th tube held a reference sample holding 0.2 g glycerin and a nitrogen-free paper. Approx 1-1.5 g of the homogenized clip-fish were weighed on nitrogen-free paper and placed into 300 mL sample tubes 4-16 together with the paper. The rest of the homogenized clip-fish were frozen again in zip-lock bags for use to determine the FAA profile. Two Titanium tablets and 15 mL con-

centrated sulphuric acid (98%) were added to each sample tube. The rack holding the sample tubes was then placed into the KjelDigester K-449. The Digester was set to have 280 °C as the start temperature, the second step was to hold 320 °C for 20 min, and the third step were set to hold 420 °C for 100 min. The fourth and last step was cooling for 35 min. After cooling, the rack was transferred to the KjelMaster system K-375/K-376 where the distillation and titration started. The parameters of KjelMaster system K-375 are listed in Table D.1.

After distillation and titration, the protein content was automatically calculated by KjelMaster K-375. The weight fraction of nitrogen (W_N) was first calculated by

$$W_N = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot M_N}{m_{Sample} \cdot 1000}, \tag{3.4}$$

where V_{Sample} refers to the volume of the titrant in the sample [mL], V_{Blank} refers to the mean volume of the titrant in the blanks [mL], z refers to the molar valence factor (= 2, for H_2SO_4), c refers to the titrant concentration [$\mathrm{mol\,L^{-1}}$], f refers to the titrant factor (= 1, for commercial solutions), m_{Sample} refers to the sample weight [g], and 1000 refers to the conversion factor [$\mathrm{mL\,L^{-1}}$]. Then the KjelMaster system calculates the percentage nitrogen content ($\%N$) and protein content ($\%P$) by

$$\%N = W_N \cdot 100\%, \tag{3.5}$$

and

$$\%P = W_N \cdot PF \cdot 100\%, \tag{3.6}$$

using the weight fraction of nitrogen and sample-specific protein factor (PF= 6.25 for fish and meat products). Glycine was used as a control, where the percentage of nitrogen content in glysine ($\%N_{Gly}$) ere calculated by

$$\%N_{Gly} = \frac{\%N \cdot 100}{P}. \tag{3.7}$$

3.2.8 Free amino acid content

The FAA profile of the clip-fish samples from days 0, 24, and 48 was determined using a method devised by Osnes and Mohr (1985). A water-soluble protein extract was made by homogenizing 2 g of the already homogenized clip-fish loins with 10 mL distilled for 50 sec at 14 500 rpm, then centrifuged for 4 min at 4800 rpm. 1 mL water-soluble protein extract was added to 1.5 mL eppendorf tube, together with 0.25 mL 10% sulphosalisylic acid and shaken vigorously. The samples were left in a fridge at 4 °C for a minimum of 30 min before they were sentrifuged for 10 min at 10000 rpm. The supernatant was diluted (1:25) with distilled water, and filtered through a 0.22 µm polyethersulfone filter (VWR International; North America) into 1.5 mL HPLC-vales. The FAA profile was measured using an ultra-HPLC (UltiMate 300, Thermo Scientific) system, including a TSP P400 pump, ultimate 3000WP injector, RF2000 detector, and a Nova-Pak C18 column (WAT086344, particle size: 4 µm, 3.9 mm×150 mm, Waters Corp., USA). Methanol and 0.08 M Sodium acetate with 2 % tetrahydrofuran were applied as mobile phases. The system's flow rate was adjusted to 0.9 mL min^{-1} (Kendler et al., 2023).

3.3 Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 28.0.1.0, including analysis of variance (one-way ANOVA), general linear modeling (GLM) (test between-subjects effects and pairwise comparisons), and post hoc Tukey's test for all the data collected in the experiment. Box plots were used to detect outliers, which were removed before further analyses. A t-test on independent samples was performed to check for any significant differences between replicate 1 and 2, for all the data obtained. Since there were no differences, replicates 1 and 2 were treated as one data set for further statistical analysis. In GLM, the days, pressure (400 or 600 MPa), treatment (HPP or SGS+HPP), and packaging (L=PA/PB and B=BioPBS), were used as fixed factors. In addition, the fillet height was used as a covariate when analyzing the results of the salt content and the textural properties. The control groups were excluded from the statistical analysis, as they were not treated with HPP, and they were only packed in PA/PE packages. However, they are present in some of the figures. All results are reported in mean ± standard error of mean (mean ± SEM). Pearson Correlations were also conducted between the different parameters.

4 Results and Discussion

The main objective of this study was to compare biobased and biodegradable food packaging made of cellulose film laminated to BioPBS to traditional food packaging made of PA/PE, by investigating if the biobased packaging could compete with the traditional packaging when clip-fish were exposed to SGS and HPP at 400 MPa and 600 MPa as pre-treatments. Table 3.1 gives a full description of the abbreviations used in the figures. As the t-test on independent samples did not show any significant differences between replicate 1 and 2 for any of the quality parameters ($0.071 < p < 0.797$), the results are based from one dataset holding them both.

4.1 Weight changes after rehydration

The resulting weight gain of the clip-fish loins from the rehydration is presented in Figure 4.1, showing the mean percentage weight change. The weight gain per hour was greatest in the first six hours, with a measured weight gain of $9.08 \pm 1.78\%$ at 6 h. After 12 h and 24 h the loins gained further $3.24 \pm 1.09\%$ and $3.77 \pm 0.45\%$ respectively. At 48 h, the clip-fish had gained $2.98 \pm 0.58\%$ more, resulting in a total percentage weight gain of $17.01 \pm 0.69\%$.

These results align with Rode and Rotabakk (2021) rehydration of clip-fish. They used the same rehydration method, which resulted in a total weight gain of $17.7 \pm 1.1\%$ (SD). They also had the highest weight change in the first six hours, with a weight gain of 7.3%. The initial low water content of the clip-fish explains the high weight gain during the first 6 hours, as the concentration difference is greatest at that point.

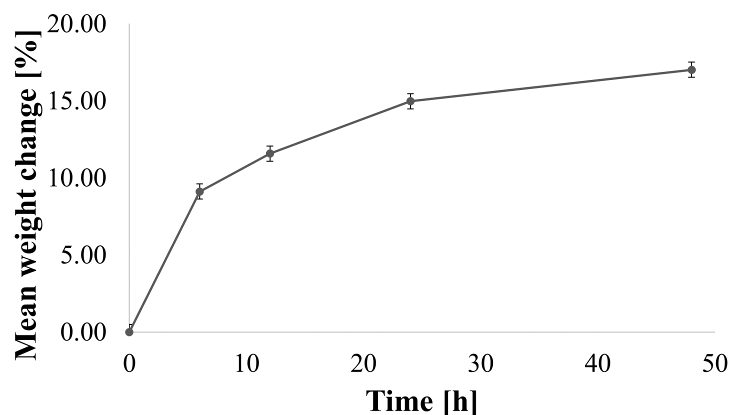


Figure 4.1: Mean percentage weight change of the clipfish loins during the rehydration period from 0 to 48 hours. The greatest weight change was after 6 h, 9.12 ± 1.80 % of its initial weight, after 48 h the clipfish loin had a total weight gain of 17.01 ± 0.69 %.

4.2 Head-space gas composition

The head-space gas composition (Table 4.1) was measured after the loins were exposed to SGS for 12 h with a mean percentage of $91.72 \pm 1.61\%$ CO_2 and $1.66 \pm 0.32\%$ O_2 for replicate 1 and $99.44 \pm 0.12\%$ CO_2 and $0.5 \pm 0.03\%$ O_2 for replicate 2. There is a significant difference between the head-space gas measured for replicate 1 and 2 ($p < 0.008$).

The significant difference in the gas composition of the head-space gas did not affect further quality measurements. This was proven by the results of the t-tests (section 4). CO_2 is denser than O_2. Thus, tilting the plates before measuring the gas composition allowed the CO_2 to be more evenly distributed within the packaging. This was done for plates 1-5 in replicate 2. However, not for replicate 1, which is the reason for the deviation between the replicates (Table 4.1).

Table 4.1: Gas composition in the head-space gas after SGS treatment and 12 h chilled storage, a mean percentage of $91.72 \pm 1.61\%$ CO_2 and $1.66 \pm 0.32\%$ O_2 for replicate 1 and $99.44 \pm 0.12\%$ CO_2 and $0.5 \pm 0.03\%$ O_2 for replicate 2.

Replicate	Plate	CO_2 [%]	O_2 [%]
1	Control 1	97.7	0.4
	Control 2	97.5	0.4
	Control 3	97.7	0.4
	1	93.1	1.4
	2	95.8	0.8
	3	88.4	2.2
	4	93.8	1.2
	5	87.5	2.5
2	Control 1	97.5	0.6
	Control 2	97.0	0.6
	Control 3	97.6	0.3
	1	99.4	0.5
	2	99.3	0.5
	3	99.4	0.5
	4	99.3	0.6
	5	99.8	0.4

4.3 Salt content

Measurements of the salt content (Figure 4.2) were only performed for day 0, to see if the different treatments had any impact on the salt content, and to evaluate the effect of the rehydration. When performing the statistical analysis of the salt content, the fillet height was used as a covariate.

There were no significant differences between the treatment groups ($p = 0.099$). However, the fillet height significantly affected the salt content ($p < 0.001$), where a thicker fillet resulted in greater salt content. The overall total mean of the salt content of the clip-fish loins was $3.32 \pm 0.17\%$.

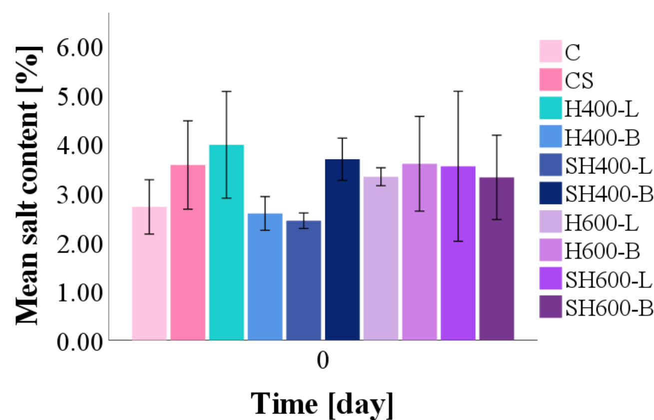


Figure 4.2: Mean salt content of the different treatment groups from day 0. The salt content did not significantly vary between the treatment groups ($p = 0.099$). However, it was significantly affected by the fillet height. The overall total mean of the salt content of the clip-fish loins was $3.32 \pm 0.13\%$.

The salt content of the clip-fish after rehydration is somewhat higher than the recommended level of 2-3% (Nofima, 2022). However, it is still lower than $3.8 \pm 1.2\%$ (SD), which Rode and Rotabakk (2021) obtained after the same rehydration method. In this study, there were large variations in the size of the clip-fish loins, leading to their high salt content deviation. Thus, for later experiments, it might be beneficial to have a standard fillet height, or sort the loins by size and expose the bigger lions for a longer rehydration period.

4.4 Microbial growth

The results of TAPC and TPC are illustrated in Figure 4.3 and 4.4 respectively, with the associated values (mean ± SEM) listed in Table E.1. The control groups (C and CS) are illustrated in

the figures. However, they were not included in the statistical analysis as they were untreated with HPP. The clip-fish were considered spoiled when the TAPC or the TPC reached 10^7 cfu g^{-1}. From both figures (4.3 and 4.4), a significant difference between C and CS can be observed ($p < 0.001$). The control group (C), which did not get any additional treatments, were soiled on day 6 with a TAPC and a TPC of 7.68 ± 0.29 log(cfu/g) and 7.46 ± 0.18 log(cfu/g), respectively. The control group treated with SGS (CS) surpassed day 10 with a TAPC and TPC of 4.83 ± 0.22 log(cfu/g) and 5.57 ± 0.46 log(cfu/g), respectively.

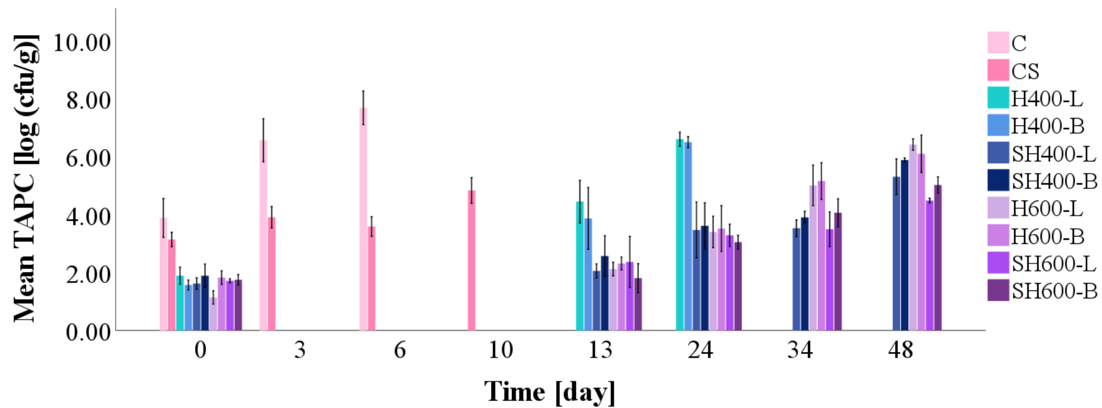


Figure 4.3: Total aerobic plate count (TAPC) throughout the experiment period, corresponding values can be found in the Table E.1. The packaging (L and B) did not significantly impact ($p = 0.567$) the TAPC. However, storage time, pressure level, and treatment did ($p < 0.001$). After day 24, H400-L and H400-B were considered spoiled. The other groups were still within the range of 7 log(cfu/g) when the experiment ended.

The TAPC (Figure 4.3) significantly differed throughout the experiment based on the storage days ($p < 0.001$), pressure (400 and 600 MPa, $p < 0.001$), and treatments (H and SH, $p < 0.001$). However, the packaging material (L and B) did not significantly impact microbial growth ($p = 0.567$). On day 24, the groups H400-L and H400-B reached 6.59 ± 0.12 log(cfu/g) and 6.49 ± 0.10 log(cfu/g) respectively. They were expected to surpass 7 log(cfu/g) before day 34. Thus, they were excluded from further analysis past day 24. All the other treatment groups did not spoil before the study ended on day 48. It is reasonable to believe that the groups SH400-L, SH400-B, SH600-L, and SH600-B would still have been in the accepted range below 7 log(cfu/g) on day 60 if the study had lasted longer. In addition, there were significant differences between the groups treated with only HPP and those additionally treated with SGS (H and SH, respectively). On day 13 and 24, there was a significant difference between the groups H400 and SH400 ($p < 0.001$), where H400 had a higher microbial growth of 1.84 ± 0.27 log(cfu/g), and 3.00 ± 0.26 log(cfu/g), respectively. On day 34 and 48, there was a significant difference

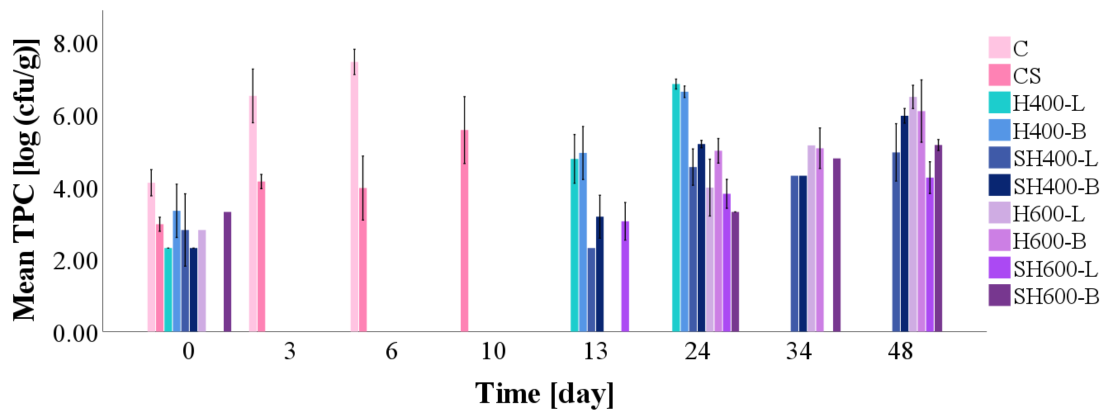


Figure 4.4: Total psycotrophic count (TPC) throughout the experiment period, corresponding values can be found in the Table E.1. The packaging (L and B) and pressure level did not significantly impact the TPC ($p = 0.169$ and $p = 0.118$ respectively). However, storage time and treatment did ($p < 0.001$). After day 24, H400-L and H400-B were considered spoiled. The other groups were still within the range of 7 log(cfu/g) when the experiment ended. However, H600-L and H600-B were close to being considered spoiled.

between the groups H600 and SH600 ($p < 0.001$), where H600 had a higher microbial growth of 1.25 ± 0.27 log(cfu/g), and 1.44 ± 0.29 log(cfu/g), respectively.

The TPC (Figure 4.4) significantly differed throughout the experiment based on the storage days ($p < 0.001$), and the treatments (H and SH, $p < 0.001$). There were, however, no differences between pressure and packaging material ($p = 0.118$ and $p = 0.169$ respectively). Just as the treatment groups H400-L and H400-B had high values of TAPC on day 24, they also had high values of TPC (6.85 ± 0.07 log(cfu/g) and 6.63 ± 0.08 log(cfu/g) respectively) which were also expected to surpass 7 log(cfu/g) before day 34. All the other groups were viable on day 48, although H600-L and H600-B would not be viable for much longer. Significant variations were observed between H400-L and H400-B ($p = 0.031$) on day 0 with a difference of 1.03 ± 0.5 log(cfu/g), and between H600-L and H600-B on day 24 ($p = 0.044$) with a difference of 1.02 ± 0.50 log(cfu/g). On day 48 there were significant variations between SH400-L and SH400-B ($p = 0.008$), and SH600-L and SH600-B ($p = 0.034$) with the variations of 1.01 ± 0.40 log(cfu/g) and 0.9 ± 0.42 log(cfu/g) respectively. Just as TAPC, there were also overall significant differences between the treatment groups H and SH. On day 13, H400 differed from SH400 ($p < 0.001$) with a difference of 2.12 ± 0.40 log(cfu/g). On day 24, there were significant differences between H400 and SH400 ($p < 0.001$), and H600 and SH600 ($p = 0.005$), with variations of 1.87 ± 0.30 log(cfu/g) and 0.93 ± 0.32 log(cfu/g) respectively.

As for HSPB, only the control groups on day 0 had any HSPB, where group C had 1.98 ± 0.20 log(cfu/g) and group CS had 1.84 ± 0.24 log(cfu/g). However, these did not significantly differ ($p = 0.648$).

The total microbial growth was significantly higher in the samples only treated with HPP than those additionally treated with SGS. Applying 600 MPa of pressure gave a significantly lower microbial growth than 400 MPa. However, the combination of SGS and HPP at 400 MPa gave a significantly lower (TAPC) or similar (TPC) microbial growth than only using HPP at 600 MPa. This supports Abel et al. (2019) conclusion of SGS having antimicrobial properties. According to Al-Nehlawi et al. (2014) HPP can enhance the effect of SGS by breaking the cell membrane, making it easier for CO_2 to dissolve. This could explain why HPP with lower pressure levels and SGS as a pre-treatment can yield equally satisfactory results as HPP with higher pressure levels without SGS. Dang et al. (2021) study on pre-cooked chicken breast treated with SGS and HPP at 600 MPa determined that the microbial shelf life of the chicken was longer for the samples only treated with HPP compared to those additionally treated with SGS, and concluded that the high pressure level surpassed the SGS effect. However, in the current study, the clip-fish treated with SGS in addition to HPP at 600 MPa had a significantly lower microbial growth at the end of the study.

4.5 pH

The results of the measured pH throughout the experiment are listed in Table 4.2. The different package materials (L and B) did not contribute to any significant variations in pH ($p = 0.673$), and neither did the different pressure levels used in the HPP (400 and 600 MPa, p=0.621). However, the storage days ($p < 0.001$) and the treatments (H and SH, $p = 0.020$) did.

There were significant differences between treatment group H400 and SH400 on day 0 ($p = 0.004$) with a mean difference of 0.09 ± 0.3, which can be explained by the acidic effect of dissolved CO_2 for those treated with SGS (Sivertsvik, Jeksrud et al., 2002; Rode and Rotabakk, 2021). The pH dropped significantly in general from day 0 to day 13, and from day 24 to day 34 for each sample group ($p < 0.001$). On day 48, there was no significant difference between the treatment groups. These results are in line with the results of Rode and Hovda (2016), which treated salmon, cod, and mackerel with HPP at 200 and 500 MPa. However, they saw an increase in pH after the first 4 of 7 days, then the pH decreased below the initial pH.

Table 4.2: Mean of pH measurement of all the treatment groups at each sample day (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively.

Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	6.77 ± 0.03^{b}	6.70 ± 0.02^{ab}	6.65 ± 0.03^{a}	6.65 ± 0.03^{a}	6.70 ± 0.02^{ab}	6.69 ± 0.02^{ab}	6.66 ± 0.02^{a}	6.64 ± 0.02^{a}	$p_D < 0.001$
13	6.49 ± 0.03	6.51 ± 0.04	6.48 ± 0.01	6.53 ± 0.01	6.53 ± 0.01	6.53 ± 0.02	6.49 ± 0.01	6.55 ± 0.02	$p_{Pr} = 0.621$
24	6.44 ± 0.05	6.48 ± 0.04	6.44 ± 0.02	6.50 ± 0.03	6.48 ± 0.05	6.50 ± 0.03	6.47 ± 0.03	6.44 ± 0.03	$p_T = 0.02$
34			6.41 ± 0.02^{ab}	6.41 ± 0.04^{ab}	6.47 ± 0.03^{b}	6.40 ± 0.05^{ab}	6.37 ± 0.05^{a}	6.39 ± 0.04^{ab}	$p_{Pa} = 0.673$
48			6.38 ± 0.02	6.39 ± 0.03	6.42 ± 0.03	6.44 ± 0.020	6.41 ± 0.03	6.46 ± 0.03	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

4.6 Drip loss

The DL significantly differed throughout the experiment based on the storage days ($p < 0.001$), as well as the different treatments (H and SH, $p < 0.001$). The groups treated with SGS had a higher DL than the groups only treated with HPP. There were, however, no differences between the pressure level of HPP and packaging material ($p = 0.280$ and $p = 0.290$, respectively).

On day 0, the DL measured were the result of the different processing treatments (Figure 4.5), while the DL measured later in the experiment period (Figure 4.6) were the result of the storage time. The corresponding values of the means can be found in Table E.2.

On day 0 (Figure 4.5), the DL of SH600-L was significantly higher than the DL of H400-L, H400-B, SH400-B, and H-600-L ($p < 0.049$). The control group C was also significantly lower than SH400-L ($p = 0.046$) and SH600-L ($p = 0.003$). However, there were no significant variations between the two control groups (C and CS, $p = 0.055$). Even though the difference was not significant, the DL was higher in the sample groups treated with SGS in addition to HPP.

The difference in DL from day 0 to day 24 and 48, were due to the storage period. The DL of all the treatment groups increased from day 0 to the end of the experiment period. However, only SH400-B and H600-L significantly increased. SH400-B significantly increased by $7.67 \pm 0.42\%$ ($p < 0.001$), while H600-L significantly increased by $3.61 \pm 0.42\%$ ($p = 0.009$). Thus, the different treatment processes were the main reason for the DL.

Rode and Rotabakk (2021) concluded that the HPP treatment of clip-fish resulted in a significantly higher DL than non-treated clip-fish, and Abel et al. (2019) determined that SGS did not contribute to any significant difference in the sous vide treated salmon, and concluded that the significant difference in DL was due to sous vide treatment prior to the SGS treatment.

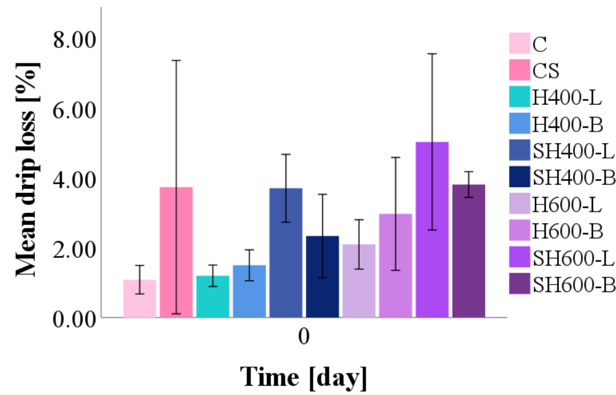


Figure 4.5: The drip loss (DL) measured on day 0 resulted from the different processing treatments. Pairwise Comparison by the general linear model showed that SH600-L had the highest DL, and were significantly higher ($p < 0.049$) than the control group C, and the treatment groups H400-L, H400-B, SH400-B, and H-600-L.

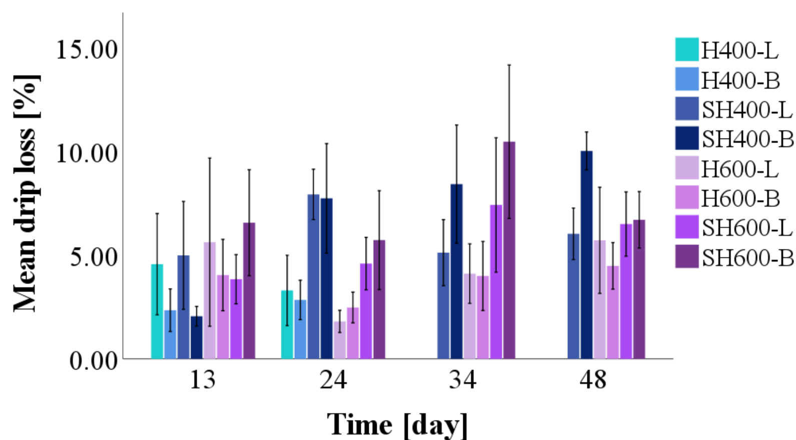


Figure 4.6: The drip loss (DL) measured on day 13, 24, 34, and 48 resulted from the storage time. Only the DL of SH400-B and H600-L significantly increased from day 0 to day 48.

4.7 Surface color

The resulting means of the surface color measurements (L*, a*, b*, chroma, and hue) are listed in Table 4.3. All the color parameters significantly differed throughout the experiment based on the storage days ($p < 0.001$). However, only the yellowness, b*, and chroma significantly

differed based on the pressure level ($p = 0.049$ and $p = 0.045$, respectively) and the packaging materials (L and B, $p = 0.017$ for both). None of the color measurements significantly differed based on the treatments (H and SH, $p > 0.099$).

The lightness, L*, had some variations between groups with different packaging materials which were exposed to the same treatment. On day 13, H400-B was significantly lighter than H400-L ($p = 0.038$) with a difference of 2.22 ± 1.07. On day 24, SH600-B was significantly lighter than SH600-L, with a difference of 2.16 ± 1.07. However, on day 34, H600-L was significantly lighter than H600-B ($p = 0.002$) with a mean difference of 3.55 ± 1.12. L* significantly differed through the storage time ($p < 0.001$). However, none of the treatment groups showed any significant changes between day 0 and 48 (day 24 for H400-L and H400-B, $p > 0.121$). The result of the lightness is in line with the results of Rode and Rotabakk (2021) study on rehydrated clip-fish treated with HPP, where they did not find any significant difference between the untreated clip-fish and the clip-fish treated with HPP at 500 MPa. They suggested that some of the pressure-sensitive protein had already been denatured during the salting and drying process. According to Christensen et al. (2017) study where fresh cod was treated with HPP 500 MPa for 2 min at 8-9 °C, the lightness increased by 20%. This emphasizes Rode and Rotabakk (2021) assumption of protein denaturation in clip-fish prior to the HPP treatment.

Redness, a*, did not significantly differ between the samples packaged in different package materials ($p = 0.392$). However, on day 24, SH600-L was significantly higher than SH600-B ($p = 0.031$) with a difference of 0.532 ± 0.245. Additionally, on day 34, H600-L was significantly lower than H600-B ($p = 0.002$) with a difference of 0.758 ± 0.245. a* significantly differed through the storage time ($p < 0.001$). However, only SH400-B significantly differed from day 0 to day 48 ($p < 0.001$), with a mean difference of 0.86 ± 0.26.

Yellowness, b*, significantly differed thought the experiment based on the different package materials ($p = 0.017$). However, an actual variation between the different packaging materials was only observed on day 13, where H400-L was significantly lower than H400-B ($p = 0.048$) with a mean difference of 1.91 ± 0.96. b* significantly differed through the storage time ($p < 0.001$). However, none of the treatment groups showed any significant changes from day 0 to day 48 ($p > 0.068$).

Table 4.3: Mean of surface color measurement of all the treatment groups at each sample day (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively.

Lightness, L*									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	94.93 ± 0.37	94.51 ± 0.23	95.03 ± 0.66	95.59 ± 0.92	95.52 ± 0.98	94.08 ± 1.11	94.72 ± 0.29	94.13 ± 1.24	$p_D < 0.001$
13	**92.18 ± 0.82^{a}**	**94.40 ± 0.76^{bc}**	93.11 ± 0.94^{ab}	94.82 ± 1.27^{bc}	94.82 ± 0.58^{bc}	95.58 ± 0.44^{bc}	94.66 ± 0.11^{bc}	95.79 ± 0.55^{c}	$p_{Pr} = 0.727$
24	96.29 ± 0.95^{c}	96.25 ± 0.51^{c}	96.70 ± 0.39^{c}	96.71 ± 0.84^{c}	94.12 ± 1.02^{ab}	96.09 ± 0.35^{abc}	**93.95 ± 0.64^{a}**	**96.11 ± 0.62^{bc}**	$p_T = 0.604$
34			93.12 ± 0.38^{a}	92.56 ± 0.32^{a}	**95.65 ± 1.36^{b}**	**92.10 ± 0.38^{a}**	92.82 ± 1.43^{a}	93.77 ± 0.53^{ab}	$p_{Pa} = 0.808$
48			95.03 ± 0.31	94.55 ± 0.41	94.91 ± 0.49	94.62 ± 0.63	95.11 ± 0.33	94.51 ± 0.60	
Redness, a*									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	-0.02 ± 0.08^{ab}	-0.27 ± 0.16^{a}	-0.24 ± 0.09^{ab}	0.22 ± 0.22^{b}	-0.48 ± 0.09^{a}	-0.14 ± 0.09^{ab}	-0.31 ± 0.10^{a}	-0.02 ± 0.13^{ab}	$p_D < 0.001$
13	-0.26 ± 0.17^{ab}	-0.19 ± 0.13^{ab}	0.18 ± 0.25^{b}	-0.10 ± 0.23^{ab}	-0.54 ± 0.14^{a}	-0.40 ± 0.16^{a}	-0.18 ± 0.07^{ab}	-0.37 ± 0.21^{a}	$p_{Pr} = 0.782$
24	-0.38 ± 0.32^{ab}	-0.53 ± 0.16^{ab}	-0.37 ± 0.22^{ab}	-0.82 ± 0.13^{a}	-0.10 ± 0.18^{bc}	-0.21 ± 0.11^{bc}	**0.28 ± 0.08^{c}**	**-0.25 ± 0.29^{b}**	$p_T = 0.099$
34			0.19 ± 0.16^{b}	-0.02 ± 0.12^{ab}	**-0.38 ± 0.28^{a}**	**0.38 ± 0.34^{b}**	0.35 ± 0.25^{b}	-0.04 ± 0.05^{ab}	$p_{Pa} = 0.392$
48			-0.35 ± 0.12	-0.64 ± 0.05	-0.41 ± 0.03	-0.49 ± 0.13	-0.32 ± 0.07	-0.49 ± 0.14	
Yelloness, b*									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	11.17 ± 0.35^{ab}	11.56 ± 0.35^{b}	10.45 ± 0.30^{ab}	11.82 ± 0.64^{b}	9.57 ± 0.37^{a}	11.10 ± 0.28^{ab}	10.70 ± 0.65^{ab}	12.20 ± 0.24^{b}	$p_D < 0.001$
13	**11.35 ± 0.10^{a}**	**13.26 ± 0.79^{b}**	12.18 ± 0.52^{ab}	12.20 ± 0.58^{ab}	11.47 ± 0.30^{a}	11.81 ± 0.25^{ab}	11.68 ± 0.55^{ab}	12.41 ± 0.35^{ab}	$p_{Pr} = 0.049$
24	12.41 ± 1.10	11.43 ± 0.37	11.65 ± 0.32	10.99 ± 0.37	12.60 ± 0.81	11.97 ± 0.68	12.39 ± 0.23	11.22 ± 0.58	$p_T = 0.944$
34			13.56 ± 0.82^{c}	13.25 ± 0.72^{bc}	11.13 ± 0.65^{a}	12.71 ± 0.40^{abc}	11.63 ± 0.26^{ab}	12.41 ± 0.29^{abc}	$p_{Pa} = 0.017$
48			11.54 ± 0.63^{ab}	12.95 ± 1.13^{b}	11.15 ± 1.15^{a}	11.34 ± 1.16^{ab}	11.17 ± 0.45^{a}	12.20 ± 0.45^{ab}	
Chroma C*									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	11.17 ± 0.35^{ab}	11.57 ± 0.34^{b}	10.45 ± 0.30^{ab}	11.83 ± 0.64^{b}	9.59 ± 0.36^{a}	11.10 ± 0.28^{ab}	10.71 ± 0.65^{ab}	12.21 ± 0.24^{b}	$p_D < 0.001$
13	**11.36 ± 0.10^{a}**	**13.27 ± 0.79^{b}**	12.20 ± 0.51^{ab}	12.21 ± 0.58^{ab}	11.49 ± 0.30^{a}	11.83 ± 0.25^{ab}	11.68 ± 0.55^{ab}	12.42 ± 0.35^{ab}	$p_{Pr} = 0.045$
24	12.51 ± 1.03	11.45 ± 0.37	11.67 ± 0.31	11.02 ± 0.37	12.61 ± 0.81	11.99 ± 0.67	12.39 ± 0.24^{ab}	11.25 ± 0.58	$p_T = 0.968$
34			13.56 ± 0.83^{c}	13.26 ± 0.71^{bc}	11.16 ± 0.64^{a}	12.74 ± 0.41^{abc}	11.64 ± 0.27^{ab}	12.41 ± 0.30^{abc}	$p_{Pa} = 0.017$
48			11.55 ± 0.63^{ab}	12.97 ± 1.13^{b}	11.16 ± 1.15^{a}	11.36 ± 1.15^{ab}	11.18 ± 0.45^{a}	12.22 ± 0.45^{ab}	
Hue, h°									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	89.18 ± 0.22^{b}	88.31 ± 0.66^{ab}	88.57 ± 0.43^{ab}	87.72 ± 0.53^{ab}	**87.00 ± 0.68^{a}**	**89.02 ± 0.36^{b}**	88.60 ± 0.29^{ab}	88.90 ± 0.34^{b}	$p_D = 0.008$
13	88.71 ± 0.31^{ab}	88.98 ± 0.38^{b}	88.73 ± 0.50^{ab}	88.34 ± 0.43^{ab}	87.34 ± 0.69^{a}	88.06 ± 0.79^{ab}	88.78 ± 0.46^{ab}	87.98 ± 0.83^{ab}	$p_{Pr} = 0.836$
24	86.96 ± 0.85^{ab}	87.01 ± 0.68^{ab}	87.37 ± 0.78^{abc}	85.89 ± 0.63^{a}	88.42 ± 0.07^{bc}	88.85 ± 0.31^{c}	**88.72 ± 0.37^{c}**	**86.98 ± 0.93^{ab}**	$p_T = 0.552$
34			88.98 ± 0.21^{bc}	89.02 ± 0.30^{bc}	86.70 ± 0.80^{a}	87.67 ± 0.51^{ab}	**87.68 ± 0.84^{ab}**	**89.56 ± 0.10^{c}**	$p_{Pa} = 0.605$
48			88.02 ± 0.45	87.41 ± 0.52	87.72 ± 0.60	87.13 ± 0.95	87.97 ± 0.16	87.61 ± 0.59	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

Chroma, C*, significantly differed thought the experiment based on the different package mater-

ials ($p = 0.017$), but just as yellowness, there was only a significant difference between H400-L and H400-B on day 13 ($p = 0.046$), with a mean difference of 1.91 ± 0.95. Chroma also significantly differed throughout the experiments' storage time ($p < 0.001$). However, none of the treatment groups showed any significant changes between day 0 and 48 (day 24 for H400-L and H400-B, $p > 0.067$).

The hue value had no significant differences between the different treatments (H and SH, $p = 0.552$), pressure levels (400 and 600 MPa), and packages (L and B, $p = 0.836$). However, on day 0, H600-L had a significantly lower hue value than H600-B ($p = 0.013$) with a difference of 2.02 ± 0.81. On day 24 and 34, SH600-L and SH600-B differed from each other ($p = 0.033$ and $P = 0.021$, respectively). However, on day 24, SH600-L was significantly higher than SH600-B with a difference of 1.70 ± 0.81. While on day 34, SH600-L was significantly lower than SH600-B with a difference of 1.88 ± 0.81. Hue also significantly differed throughout the experiments' storage time ($p = 0.008$). However, only H400-L and H600-B significantly differed between day 0 and 48 (day 24 for H400-L, $p = 0.010$ and $p = 0.020$, respectively), with the mean differences of 2.21 ± 0.85 and 1.90 ± 0.81, respectively.

The surface color of the clipfish in Rode and Rotabakk (2021) study had no significant differences between their different treatment groups. However, the color parameter, L*, a*, and b*, all showed significantly different values with increasing storage time, where the samples became less white, more reddish, and more yellowish in color as compared to day 0.

4.8 Textural properties

The texture was evaluated based on the clip-fish firmness (Figure 4.7) and breaking force (Figure 4.7). The associated values (mean ± SEM) are listed in Table E.3. The fillet height was used as a covariate when analyzing the results.

The firmness of the clip-fish loins did not significantly differ throughout the experiment based on the storage days ($p = 0.129$), nor did the different package materials (L and B, $p = 0.194$). However, the different treatments (H and SH, $p = 0.002$) and the different pressure levels used in HPP (400 and 600 MPa, $p = 0.006$) significantly impacted the clip-fish firmness. On day 24, there was a significant difference between the groups H400 and SH400 ($p = 0.031$), and between the groups H600 and SH600 ($p = 0.015$). Additionally, on day 34 and 48, there was a significant difference between H600 and SH600 ($p < 0.042$). The clip-fish treated with only

HPP had a significantly firmer texture than those treated with additional SGS, indicating the CO_2 loosening effect on the fish muscle.

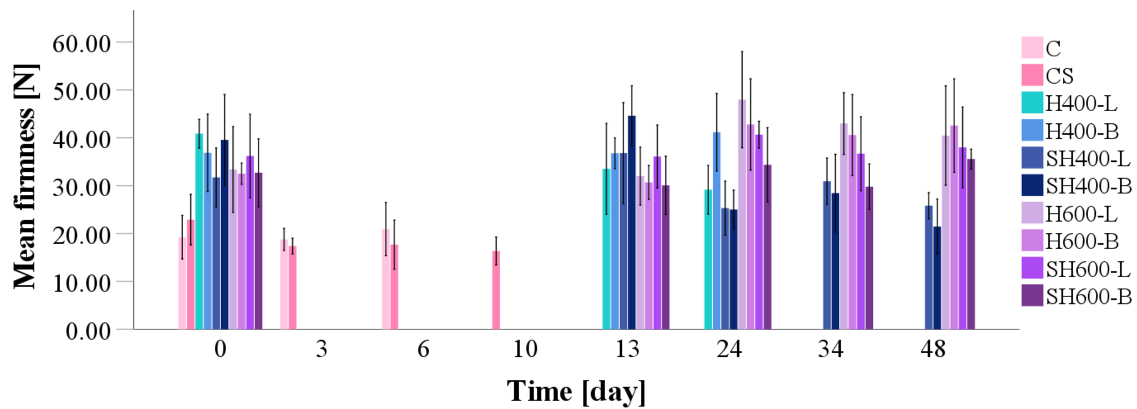


Figure 4.7: Mean firmness at 60% compression measured throughout the experiment period.

The breaking force did not significantly differ throughout the experiment based on the storage days ($p = 0.092$), nor did the different package materials (L and B, $p = 0.935$). However, the different treatments (H and SH, $p < 0.001$) and the different pressure levels used in HPP (400 and 600 MPa, $p < 0.001$) did. Like the firmness, the breaking force also had significant differences between the groups treated with only HPP and those additionally treated with SGS (H and SH, respectively). On day 24, there was a significant difference between the groups H400 and SH400 ($p = 0.022$), and between the groups H600 and SH600 ($p = 0.002$). Additionally, on day 34 and 48, there was a significant difference between H600 and SH600 ($p < 0.001$). This corresponds with the firmness of the clip-fish, as the clip-fish treated with only HPP required a significantly higher breaking force than those treated with additional SGS.

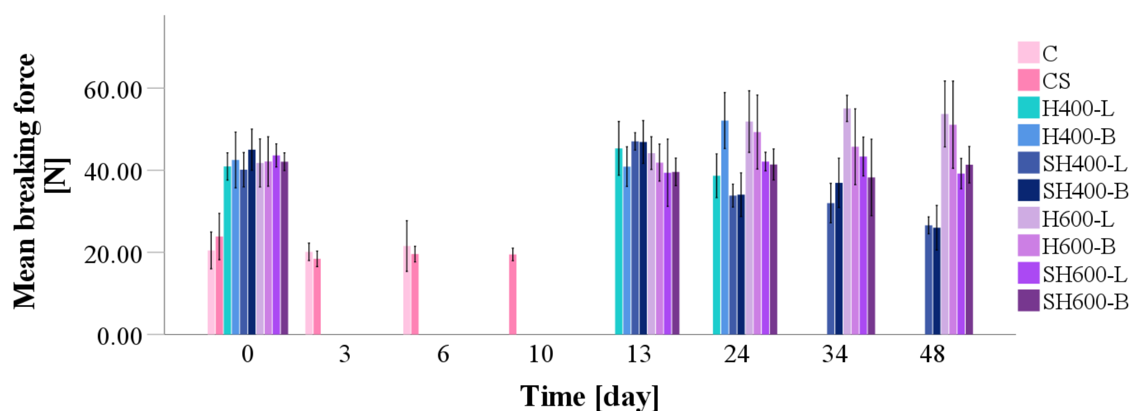


Figure 4.8: Mean breaking force measured throughout the experiment period.

4.9 Water- and protein content

The resulting percentage means of the water- and protein content are listed in Table 4.3. The water content did not significantly differ throughout the experiment based on the packaging materials (L and B, $p = 0.859$). However, the storage time ($p < 0.001$), the different pressure levels (400 and 600 MPa, $p = 0.008$), and the different treatments (H and HS, $p < 0.001$) did. A significant difference was observed only on day 0 between H400-L and H400-B, where H400-L had a significantly lower water content than H400-B ($p = 0.006$) with a difference of $1.64 \pm 0.59\%$. The water content significantly differed between the groups treated with only HPP and those additionally treated with SGS (H and SH, respectively). On day 0, 13, and 48, H600 had a significantly higher water content than SH600 ($p < 0.036$). This also applies to H400 and SH400 on day 13 ($p < 0.001$). However, the difference decreased with the storage time, where the biggest differences measured were between day 0 and day 13. After day 13, and throughout the experiment period, the water content was somewhat stable.

Table 4.4: Means of water- and protein content measurement of all the treatment groups (n=6, in each treatment group). The water content was measured on each sample day, while the protein content was measured from frozen samples taken on day 0, 24, and 48. H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively,

Water content [%]									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	**78.07 ± 0.67**^{ab}	**79.71 ± 0.50**^{d}	79.17 ± 0.21^{bcd}	78.58 ± 0.41^{abc}	79.82 ± 0.26^{d}	79.45 ± 0.40^{cd}	77.65 ± 0.37^{a}	78.11 ± 0.46^{ab}	$p_D < 0.001$
13	79.01 ± 0.32^{d}	78.07 ± 0.33^{bcd}	77.47 ± 0.43^{ab}	76.60 ± 0.15^{a}	78.68 ± 0.47^{cd}	77.78 ± 0.55^{bcd}	76.44 ± 0.44^{a}	77.60 ± 0.45^{abc}	$p_{Pr} = 0.008$
24	78.28 ± 0.68^{b}	78.09 ± 0.08^{b}	77.23 ± 0.33^{ab}	77.86 ± 0.55^{ab}	77.72 ± 0.36^{ab}	77.32 ± 0.37^{ab}	76.84 ± 0.24^{a}	77.62 ± 0.28^{ab}	$p_T < 0.001$
34			77.60 ± 0.25^{b}	77.34 ± 0.23^{ab}	77.46 ± 0.13^{ab}	76.81 ± 0.07^{ab}	76.41 ± 0.59^{a}	76.86 ± 0.31^{ab}	$p_{Pa} = 0.859$
48			77.42 ± 0.46^{ab}	78.07 ± 0.44^{b}	77.84 ± 0.50^{b}	77.53 ± 0.24^{ab}	77.19 ± 0.45^{ab}	76.42 ± 0.27^{a}	
Protein content [%]									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	18.43 ± 0.34^{a}	18.79 ± 0.36^{abc}	18.54 ± 0.23^{ab}	18.58 ± 0.25^{ab}	17.98 ± 0.55^{a}	18.59 ± 0.15^{ab}	19.41 ± 0.32^{c}	19.29 ± 0.20^{bc}	$p_D = 0.476$
24	17.92 ± 0.08^{a}	18.51 ± 0.31^{ab}	18.54 ± 0.25^{ab}	19.02 ± 0.10^{bc}	19.08 ± 0.30^{bc}	18.63 ± 0.40^{ab}	19.72 ± 0.15^{c}	19.52 ± 0.21^{c}	$p_{Pr} = 0.001$
48			18.81 ± 0.19^{a}	19.07 ± 0.31^{ab}	18.55 ± 0.39^{a}	18.43 ± 0.35^{a}	19.15 ± 0.27^{ab}	19.79 ± 0.26^{b}	$p_T < 0.001$
									$p_{Pa} = 0.169$

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

In Huss (1995), the water content of fresh cod was estimated to be in the range of $78-83\%$, and Bisenius et al. (2019) measured the water content in fresh cod to be $79.4\pm0.9\%$. The water content of the control groups (C and CS) in this study was $79.51\pm0.32\%$ and $79.62\pm0.61\%$. The water content at the beginning of this study was similar to what is commonly found in the literature. Only SH600-L and SH600-B had a significantly lower water content than the control groups C and CS.

The protein content did not significantly differ throughout the experiment based on the packaging materials (L and B, $p=0.169$), nor the storage time ($p=0.748$). However, it did significantly differ between the different treatments (H and HS, $p<0.001$), and pressure level (400 and 600 MPa, $p=0.001$). SH600 had a significantly higher protein content than H600 on all the storage days ($p<0.012$). According to a study by Bisenius et al. (2019), the protein content of fresh cod is $19.6\pm0.9\%$. Thus, protein content measured in rehydrated clip-fish does not deviate much from fresh cod.

4.10 Free amino acids content

The resulting FAA content (mean ± SEM) is listed in Table 4.5, 4.6, 4.7, and E.5, which is sorted by the different flavors associated with the different FAA (Kendler et al., 2023; Hayashi et al., 1981; Li et al., 2009; Rodríguez-González et al., 2020). Table 4.5 contains the FAA associated with sweetness, Table 4.6 contains the FAA associated with bitterness, Table 4.7 contains the FAA associated with acidity, and Table E.5 contains the FAA which are not associated with any flavor.

The FAA content associated with sweetness (Table 4.5) significantly differed throughout the experiment based on the storage time ($p<0.001$), the different pressure levels (400 and 600 MPa, $p<0.001$), and the different treatments (H and HS, $p<0.001$). There were no significant differences between the different packaging materials ($p=0.757$). However, on day 48, SH400-L contained significantly more FAA associated with sweetness than SH400-B ($p=0.023$) with a mean difference of 33.26 ± 14.15 mg/(100g fish). FAA associated with sweetness significantly increased in several of the treatment groups from day 0 to day 48 ($p<0.002$). SH400-L, SH400-B, SH600-L, and SH600-B increased with a mean difference of 186.33 ± 22.48mg/(100g fish), 129.53 ± 22.48mg/(100g fish), 48.16 ± 22.48mg/(100g fish), and 51.33 ± 22.48mg/(100g fish) respectively.

The FAA content associated with bitterness (Table 4.6) significantly differed throughout the experiment based on the storage time ($p < 0.001$), the different pressure levels (400 and 600 MPa, $p < 0.001$), and the different treatments (H and HS, $p < 0.001$). However, there were no significant differences between the different packaging materials ($p = 0.452$). FAA associated with bitterness significantly increased in several of the treatment groups from day 0 to day 48. SH400-L, SH400-B, SH600-L, and SH600-B increased with a mean difference of 171.05 ± 14.46mg/(100g fish), 161.64 ± 14.46mg/(100g fish), 44.73 ± 14.46mg/(100g fish), and 72.68 ± 14.46mg/(100g fish) respectively.

The FAA content associated with acidity (Table 4.7) significantly differed throughout the experiment based on the storage time ($p < 0.001$), the different pressure levels (400 and 600 MPa, $p < 0.001$), and the different treatments (H and HS, $p < 0.001$). However, there were no significant differences between the different packaging materials ($p = 0.713$). FAA associated with acidity significantly increased in several of the treatment groups from day 0 to day 48. SH400-L, SH400-B, SH600-L, and SH600-B increased with a mean difference of 107.67 ± 7.91mg/(100g fish), 99.04 ± 7.91mg/(100g fish), 20.37 ± 7.91mg/(100g fish), and 33.73 ± 7.91mg/(100g fish) respectively.

Table 4.5: Mean measurement of the free amino acids distribution [mg/100g fish] associated with sweet flavor of all the treatment groups from frozen samples taken on day 0, 24, and 48 (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package materials, PA/PE and BioPBS, respectively,

Treatment		H400-		SH400-		H600-		SH600-		
Amino acid	Day	L	B	L	B	L	B	L	B	p-value[1]
Ala	0	5.20 ± 0.41	6.22 ± 0.72	5.68 ± 0.44	6.13 ± 0.60	5.95 ± 0.53	6.18 ± 0.78	6.46 ± 1.03	7.36 ± 0.49	$p_D = p_{Pr} = p_T < 0.001$
	24	9.98 ± 0.85^a	9.54 ± 0.65^a	25.07 ± 1.59^b	22.20 ± 3.34^b	9.56 ± 0.61^a	9.50 ± 0.80^a	$\mathbf{23.74 \pm 3.64}^b$	$\mathbf{13.78 \pm 0.22}^a$	$p_{Pa} = 0.514$
	48			51.81 ± 6.01^c	47.15 ± 8.26^c	10.88 ± 1.96^a	11.05 ± 0.60^a	24.85 ± 0.78^b	28.40 ± 3.74^b	
Gly/Arg*	0	15.37 ± 0.13	17.58 ± 1.63	12.47 ± 0.50	15.98 ± 2.34	14.21 ± 1.50	13.74 ± 1.97	13.50 ± 1.67	14.93 ± 0.91	$p_D = p_{Pr} = p_T < 0.001$
	24	20.28 ± 1.17^a	20.02 ± 1.33^a	44.67 ± 2.44^c	38.55 ± 5.96^{bc}	22.19 ± 1.89^a	20.00 ± 1.69^a	$\mathbf{42.11 \pm 6.28}^c$	$\mathbf{26.43 \pm 1.41}^{ab}$	$p_{Pa} = 0.726$
	48			89.18 ± 9.06^c	82.49 ± 12.70^c	19.94 ± 4.93^a	22.49 ± 1.31^a	43.08 ± 1.13^b	51.1 ± 6.66^b	
Thr	0	6.11 ± 0.17	6.34 ± 0.52	4.93 ± 0.37	5.72 ± 0.68	4.36 ± 0.35	4.12 ± 0.49	5.20 ± 0.59	5.37 ± 0.50	$p_D = p_{Pr} = p_T < 0.001$
	24	7.63 ± 0.32^a	7.66 ± 0.59^a	15.30 ± 0.86^c	13.86 ± 2.03^c	7.12 ± 0.39^a	6.60 ± 0.53^a	12.08 ± 1.62^{bc}	9.99 ± 0.93^{ab}	$p_{Pa} = 0.787$
	48			27.91 ± 3.32^c	25.77 ± 3.26^c	6.83 ± 1.62^a	7.59 ± 0.36^a	12.70 ± 0.81^b	15.04 ± 1.55^b	
Ser	0	4.01 ± 0.50	4.26 ± 0.46	3.53 ± 0.37	3.99 ± 0.67	2.71 ± 0.28	3.19 ± 0.23	3.14 ± 0.56	3.54 ± 0.38	$p_D = p_{Pr} = p_T < 0.001$
	24	8.37 ± 1.24^a	6.71 ± 0.82^a	21.73 ± 1.50^b	19.29 ± 2.92^b	10.93 ± 3.03^a	5.50 ± 0.36^a	$\mathbf{18.89 \pm 2.73}^b$	$\mathbf{11.65 \pm 0.78}^a$	$p_{Pa} = 0.620$
	48			43.32 ± 4.97^c	42.24 ± 7.28^c	7.75 ± 1.98^a	6.90 ± 0.32^a	18.23 ± 1.93^b	24.25 ± 3.04^b	
Mean sweet	0	26.51 ± 4.17	30.98 ± 3.55	28.17 ± 2.56	33.30 ± 3.42	28.96 ± 3.02	24.74 ± 2.98	27.46 ± 3.42	35.39 ± 3.23	$p_D = p_{Pr} = p_T < 0.001$
FAA	24	48.31 ± 11.61 a	49.56 ± 4.73 a	96.07 ± 13.57 b	85.23 ± 19.04 ab	64.70 ± 16.70 ab	45.87 ± 4.17 a	78.38 ± 13.50 ab	87.28 ± 36.40 ab	$p_{Pa} = 0.757$
	48			214.50 ± 24.23 c	162.83 ± 32.83 b	50.99 ± 13.86 a	76.07 ± 20.92 a	67.38 ± 15.30 a	83.55 ± 20.89 a	

[*] Arginine and glycine could not be separated

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

Table 4.6: Mean measurement of the free amino acids distribution [mg/100g fish] associated with a bitter taste of all the treatment groups from frozen samples taken on day 0, 24, and 48 (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package materials, PA/PE and BioPBS, respectively,

Treatment		H400-		SH400-		H600-		SH600-		
Amino acid	Day	L	B	L	B	L	B	L	B	p-value[1]
Leu	0	3.90 ± 0.32	4.60 ± 0.38	4.51 ± 0.52	4.90 ± 0.69	3.83 ± 0.33	3.73 ± 0.49	4.55 ± 0.72	5.25 ± 0.50	$p_D = p_{Pr} = p_T < 0.001$
	24	9.90 ± 1.28^{a}	7.74 ± 0.75^{a}	30.76 ± 2.00^{c}	27.0 ± 34.30^{c}	7.58 ± 0.35^{a}	6.93 ± 0.38^{a}	24.79 ± 3.76^{bc}	15.12 ± 0.79^{ab}	$p_{Pa} = 0.534$
	48			66.54 ± 8.79^{c}	61.57 ± 11.24^{c}	9.59 ± 1.69^{a}	8.48 ± 0.52^{a}	24.88 ± 3.44^{b}	31.28 ± 4.83^{b}	
Val	0	2.40 ± 0.04	2.65 ± 0.27	2.18 ± 0.13	2.69 ± 0.37	2.16 ± 0.23	2.14 ± 0.25	2.20 ± 0.28	2.53 ± 0.18	$p_D = p_{Pr} = p_T < 0.001$
	24	5.62 ± 0.67^{a}	4.44 ± 0.44^{a}	18.02 ± 1.07^{c}	14.86 ± 2.33^{c}	4.21 ± 0.33^{a}	3.64 ± 0.24^{a}	13.62 ± 2.20^{bc}	7.98 ± 0.54^{ab}	$p_{Pa} = 0.531$
	48			40.77 ± 5.34^{c}	37.44 ± 6.92^{c}	5.61 ± 1.11^{a}	4.55 ± 0.30^{a}	15.20 ± 2.32^{b}	19.52 ± 3.04^{b}	
Ile	0	2.39 ± 0.06	2.60 ± 0.06	2.42 ± 0.21	2.54 ± 0.33	2.05 ± 0.19	2.01 ± 0.19	2.07 ± 0.16	2.64 ± 0.19	$p_D = p_{Pr} = p_T < 0.001$
	24	4.45 ± 0.48^{ab}	3.68 ± 0.23^{a}	10.39 ± 0.59^{c}	9.16 ± 1.31^{c}	3.88 ± 0.61^{a}	3.05 ± 0.17^{a}	7.40 ± 1.03^{bc}	4.93 ± 0.06^{ab}	$p_{Pa} = 0.518$
	48			21.81 ± 2.21^{c}	20.23 ± 3.55^{c}	3.78 ± 0.52^{a}	3.76 ± 0.08^{a}	8.47 ± 0.34^{b}	9.46 ± 1.27^{b}	
Met	0	1.67 ± 0.19	2.65 ± 0.29	2.53 ± 0.26	2.53 ± 0.51	1.93 ± 0.21	2.24 ± 0.29	2.29 ± 0.26	2.54 ± 0.33	$p_D = p_{Pr} = p_T < 0.001$
	24	4.77 ± 0.58^{a}	3.84 ± 0.55^{a}	15.25 ± 1.25^{c}	12.25 ± 2.32^{bc}	3.70 ± 0.30^{a}	3.15 ± 0.32^{a}	12.16 ± 2.21^{bc}	7.12 ± 0.82^{ab}	$p_{Pa} = 0.333$
	48			33.03 ± 3.55^{c}	29.55 ± 6.04^{c}	4.61 ± 0.99^{a}	3.71 ± 0.21^{a}	12.03 ± 1.64^{b}	14.56 ± 2.62^{b}	
Phe	0	2.05 ± 0.12	2.44 ± 0.10	2.23 ± 0.15	2.50 ± 0.29	1.88 ± 0.13	1.98 ± 0.17	2.10 ± 0.18	2.52 ± 0.17	$p_D = p_{Pr} = p_T < 0.001$
	24	5.22 ± 0.58^{a}	4.18 ± 0.30^{a}	13.93 ± 0.87^{c}	13.09 ± 1.94^{c}	3.99 ± 0.32^{a}	3.37 ± 0.23^{a}	10.06 ± 1.55^{bc}	6.15 ± 0.31^{ab}	$p_{Pa} = 0.717$
	48			26.45 ± 2.08^{c}	28.03 ± 4.99^{c}	4.70 ± 0.88^{a}	4.53 ± 0.08^{a}	10.66 ± 1.24^{b}	13.32 ± 2.07^{b}	
Mean bitter	0	9.54 ± 2.28	13.67 ± 1.42	13.14 ± 0.82	15.17 ± 2.12	11.85 ± 1.01	12.10 ± 1.34	13.22 ± 1.55	15.48 ± 1.24	$p_D = p_{Pr} = p_T < 0.001$
FAA	24	29.96 ± 3.48^{a}	23.88 ± 2.20^{a}	88.35 ± 5.70^{b}	76.40 ± 12.14^{b}	20.12 ± 2.87^{a}	20.15 ± 1.30^{a}	**68.04 ± 10.63** ^{b}	**34.78 ± 5.36** ^{a}	$p_{Pa} = 0.452$
	48			184.19 ± 18.52^{d}	176.81 ± 32.67^{d}	28.29 ± 5.15^{a}	23.65 ± 2.14^{a}	**57.95 ± 14.28** ^{b}	**88.16 ± 13.73** ^{c}	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

Table 4.7: Mean measurement of the free amino acids distribution [mg/100g fish] associated with acidic flavor of all the treatment groups from frozen samples taken on day 0, 24, and 48 (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package materials, PA/PE and BioPBS, respectively,

Treatment		H400-		SH400-		H600-		SH600-		
Amino acid	Day	L	B	L	B	L	B	L	B	p-value[1]
Glu	0	4.78 ± 0.27	5.20 ± 0.49	4.54 ± 0.41	5.11 ± 0.52	4.02 ± 0.38	4.33 ± 0.50	4.03 ± 0.61	4.59 ± 0.26	$p_D = p_{Pr} = p_T < 0.001$
	24	9.45 ± 0.54^{ab}	9.30 ± 1.07^{ab}	20.21 ± 1.75^{c}	19.36 ± 2.84^{c}	9.56 ± 0.76^{ab}	7.20 ± 0.30^{a}	15.84 ± 1.74^{bc}	10.12 ± 0.73^{ab}	$p_{Pa} = 0.679$
	48			47.27 ± 5.93^{d}	44.12 ± 7.58^{d}	9.42 ± 2.25^{ab}	9.36 ± 0.78^{a}	16.92 ± 0.45^{bc}	19.26 ± 1.97^{c}	
Asp	0	2.93 ± 0.20	3.11 ± 0.27	3.05 ± 0.66	3.73 ± 0.53	2.10 ± 0.25	2.09 ± 0.17	2.18 ± 0.38	2.76 ± 0.42	$p_D = p_T < 0.001$
	24	8.70 ± 0.83^{a}	8.12 ± 0.80^{a}	23.16 ± 1.26^{b}	21.92 ± 3.23^{b}	7.62 ± 1.06^{a}	5.23 ± 0.55^{a}	7.75 ± 0.62^{a}	5.39 ± 0.38^{a}	$p_{Pr} = 0.002$
	48			51.89 ± 5.43^{c}	49.30 ± 8.55^{c}	6.38 ± 1.76^{a}	6.86 ± 0.83^{a}	11.19 ± 1.45^{ab}	14.31 ± 2.35^{b}	$p_{Pa} = 0.795$
His	0	0.63 ± 0.05	0.72 ± 0.12	0.75 ± 0.13	0.72 ± 0.09	0.49 ± 0.06	0.54 ± 0.09	0.73 ± 0.20	0.66 ± 0.05	$p_D = p_{Pr} = p_T < 0.001$
	24	2.45 ± 0.36^{a}	1.86 ± 0.31^{a}	7.84 ± 0.65^{c}	6.73 ± 1.14^{c}	3.04 ± 0.90^{a}	1.20 ± 0.11^{a}	6.38 ± 1.15^{bc}	3.79 ± 0.59^{ab}	$p_{Pa} = 0.417$
	48			16.32 ± 2.08^{c}	15.18 ± 2.77^{c}	1.89 ± 0.56^{a}	1.54 ± 0.08^{a}	5.82 ± 0.92^{b}	8.16 ± 1.34^{b}	
Mean acidic	0	6.94 ± 1.45	9.03 ± 0.83	7.82 ± 1.21	9.56 ± 1.10	6.54 ± 0.65	6.62 ± 0.56	6.95 ± 1.07	8.01 ± 0.68	$p_D = p_{Pr} = p_T < 0.001$
FAA	24	17.57 ± 2.93^{ab}	19.28 ± 2.09^{ab}	51.21 ± 3.42^{c}	48.02 ± 7.17^{c}	20.23 ± 2.47^{ab}	12.44 ± 0.95^{a}	29.98 ± 3.01^{b}	19.30 ± 1.29^{ab}	$p_{Pa} = 0.713$
	48			115.49 ± 13.11^{c}	108.60 ± 18.72^{c}	16.63 ± 4.02^{a}	17.76 ± 1.52^{a}	27.32 ± 4.81^{ab}	41.74 ± 4.80^{b}	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

As the amount of FAA associated with flavor increased significantly from day 0 to day 48, it is reasonable to believe these will lead to more off-flavor than greater taste. The highest increase can be observed in the groups treated with SGS in addition to HPP. Thus, CO_2 might have contributed to the dissociation of proteins to their FAA.

4.11 Protein denaturation

The protein denaturation was measured in frozen samples from day 0, using DSC with n=6 for each treatment group. However, the sample groups treated with HPP at 600 MPa were not measured. The measurements were so uneven within each treatment group (Figure 4.9, 4.10, and 4.11) that calculating a mean from them would not have given a clear picture. Under the assumption that the treatment groups treated with HPP at 600 MPa would not give any clearer results than the treatment groups treated with HPP with 400 MPa, they were not analyzed.

The black, brown, red, and blue lines of the control group (Figure 4.9a) had an endothermal peak between 40 °C and 45 °C, which indicates protein denaturation of myosin. All the lines had an endothermal peak around 65 °C, which indicates protein denaturation of actin. The control group treated with SGS (Figure 4.9b) shows less denaturation of myosin, but there was still some denaturation of actin. This means that even though the clip-fish before the experiment was highly processed by drying and salting, the proteins were not fully denatured. However, after treating the clip-fish with HPP, the DSC did not give sufficient results to analyze (Figure 4.10 and 4.11). Dagbjørn Skipnes (Senior scientist at Nofima Stavanger) said he had experienced similar results with other products treated with HPP. However, he had not come across such results in the literature.

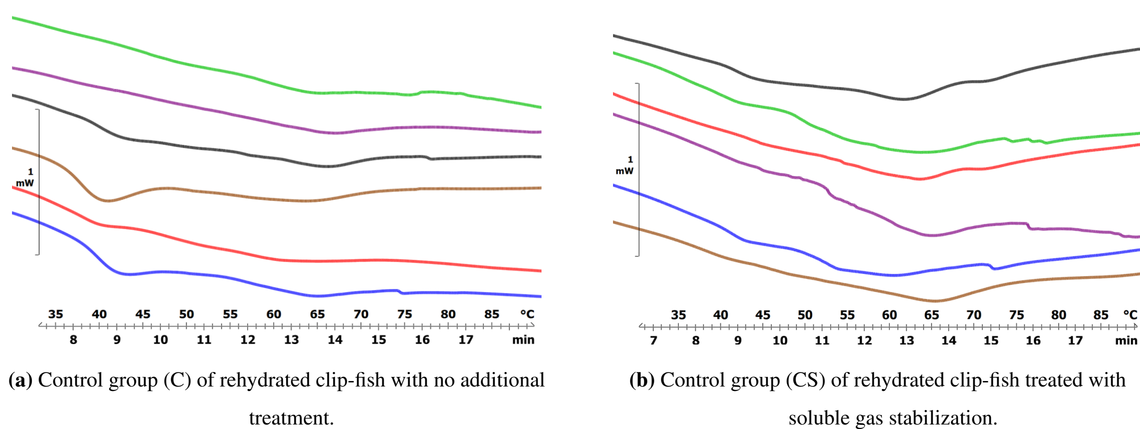
Dagbjørn Skipnes, Senior scientist Nofima Stavanger, 17.03.2023

"I have experienced disturbances, similar to this, during DSC measurements of products treated with HPP, but have not come across such results in the literature."

Christensen et al. (2017) measured the protein denaturation in fresh cod, salmon, and mackerel treated with HPP at 200 and 500 MPa where they observed that enthalpy of myosin significantly decreased after treating cod and mackerel with 200 MPa, and at 500 MPa the high pressure had already induced protein denaturation. Thus, no further protein denaturation was observed in the DSC measurement. Clip-fish is already highly processed. Di Luccia et al. (2005) investigated the effect of the hydration process on water-soluble proteins in stockfish and salted cod.

They believed that the high salt concentration in these products led to salt diffusion into the fish muscle tissue, further leading to the denaturation of sarcoplasmic- and myofibrillar proteins. Thorarinsdottir et al. (2001) investigated the changes in myofibrillar proteins during the processing of salted cod, where they observed a significant decrease in the peak size after dry salting the fresh cod, and further decrease after rehydration.

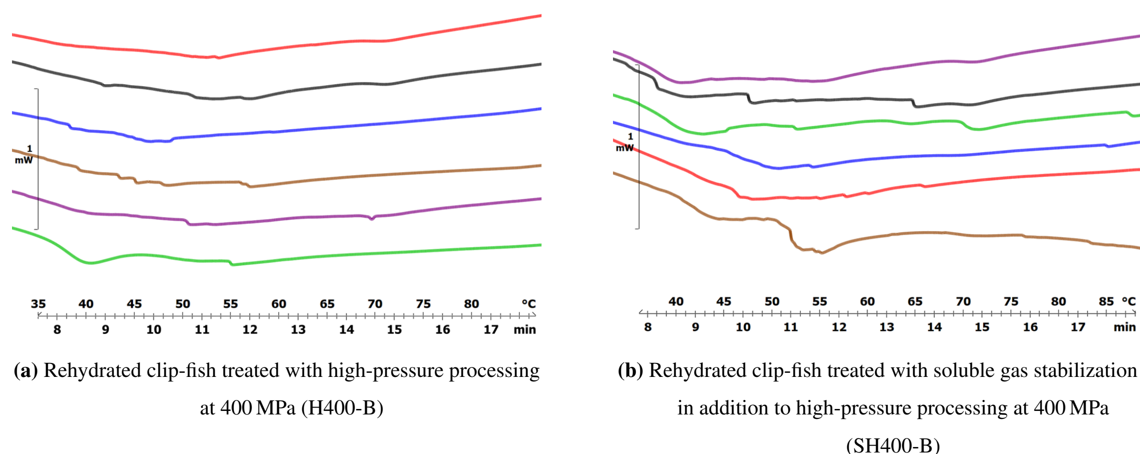
Based on the discovered information, it appears that the clip-fish protein underwent significant denaturation during its production process, and got even furthr induced when treated with HPP, which led to inconclusive results.



(a) Control group (C) of rehydrated clip-fish with no additional treatment.

(b) Control group (CS) of rehydrated clip-fish treated with soluble gas stabilization.

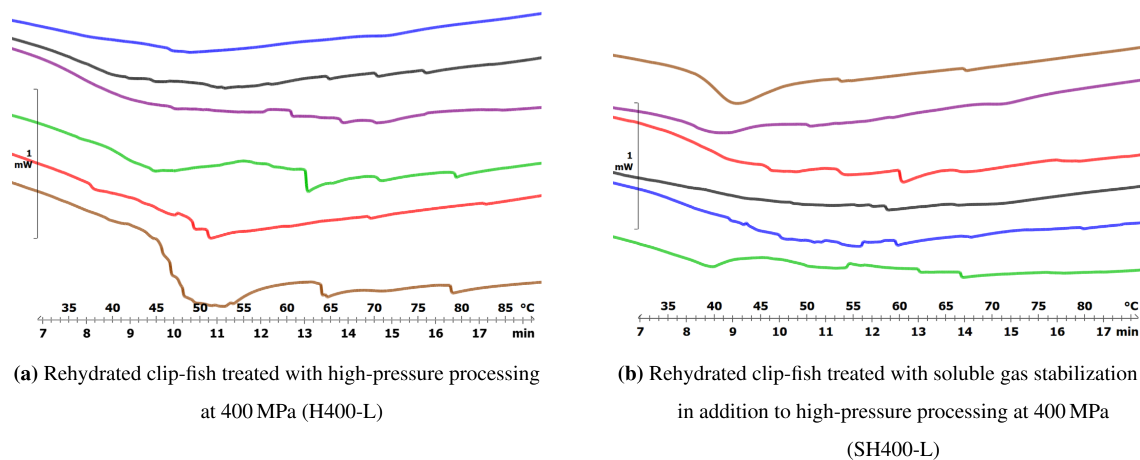
Figure 4.9: Differential scanning calorimetry (DSC) thermograms of the rehydrated clip-fish control groups (C and CS), which were not treated with high-pressure processing.



(a) Rehydrated clip-fish treated with high-pressure processing at 400 MPa (H400-B)

(b) Rehydrated clip-fish treated with soluble gas stabilization in addition to high-pressure processing at 400 MPa (SH400-B)

Figure 4.10: Differential scanning calorimetry (DSC) thermograms of rehydrated clip-fish treated with high-pressure processing at 400 MPa and packaged in PA/PE vacuum bags. The thermograms had many disturbances, which led to inconclusive results.



(a) Rehydrated clip-fish treated with high-pressure processing at 400 MPa (H400-L)

(b) Rehydrated clip-fish treated with soluble gas stabilization in addition to high-pressure processing at 400 MPa (SH400-L)

Figure 4.11: Differential scanning calorimetry (DSC) thermograms of rehydrated clip-fish treated with high-pressure processing at 400 MPa and packaged in BioPBS vacuum bags. The thermograms had many disturbances, which led to inconclusive results.

4.12 BioPBS vs. PA/PE packaging material

The only quality parameter that indicated any significant differences between the packaging materials was the surface color parameters yellowness, b*, and the color purity, C*. However, it was only on day 13 within treatment group H400, that there were significant differences between the packaging materials. None of the other quality parameters monitored in this study showed any significant differences in the clip-fish quality or microbial shelf life between the different packaging materials. Hence, the packaging made of cellulose and BioPBS was found to be as effective as PA/PE as packaging material for products treated with HPP and SGS, indicating that neither the HPP treatments nor the SGS treatment caused any significant damage to the biodegradable packaging material.

4.13 Correlations between the quality parameters

After conducting a Pearson Correlation analysis on the measured parameters (Table E.6), it is necessary to highlight some notable correlations.

- The DL negatively correlates to the fillet height, texture parameters, salt concentration, and pH. Additionally, it positively correlates to the FAA.
- The water content negatively correlates to the color parameters, fillet height, texture parameters, protein content, and the FAA.

- The pH negatively correlates to microbial growth (TAPC and TPC), and FAA.
- The microbial growth of TAPC and TPC positively correlates, however, only TAPC positively correlates to FAA.
- The fillet height negatively correlates with the DL and the water content. Additionally, it positively correlates to the salt content.

Many of these correlations are connected in a bigger picture. Protein will degrade as bacterial growth increases during storage. However, there were no significant variations in the protein content in this study. This can be explained by the method used to measure the protein content. The Kjeldahl method calculates the protein content based on the nitrogen content, which cannot distinguish between nitrogen bound to FAA and the nitrogen found in protein-bound amino acids. Due to the significant increase of FAA during the storage time, degradation of protein can be assumed (Antoine et al., 2001). Protein denaturation and degradation cause increased DL, as the free- and immobilized water of the muscle gets released. This will also cause poorer texture (Skipnes et al., 2007).

5 Conclusion

The various quality analyses revealed minimal distinctions between the packaging materials. The biodegradable and bio-based bags provided an equivalent level of protection when exposed to SGS and HPP as the non-recyclable PA/PE bags. Thus, biodegradable food packaging made of cellulose film laminated to BioPBS is an excellent alternative for the food industry, which uses SGS and/or HPP treatments.

Both SGS and HPP contributed to the extension of the microbial shelf life of the rehydrated clip-fish where HPP at 600 MPa in combination with SGS resulted in the lowest microbial growth. However, the combination of SGS and HPP at 400 MPa gave a significantly lower (TAPC) or similar (TPC) microbial growth than only using HPP at 600 MPa. Thus, by combining SGS and HPP, the pressure level used in HPP can be reduced and still maintain the desired microbial shelf life. The salting and drying of the clip-fish caused protein denaturation prior to the SGS and HPP treatment. Protein denaturation lead to lighter color in the fish muscle, which explains why there were no significant variations in the surface color between the treatment groups on day 0, as most of the protein was already denatured. The textural properties did not show any significant differences between the treatment groups on day 0. However, the differences became more significant with the storage time, where the clip-fish only treated with HPP became firmer, while the clip-fish treated with SGS in addition to HPP became softer. Thus SGS may contribute to softening the fish muscle. The FAA significantly differed throughout the experiment, where the clip-fish treated with SGS and HPP had a significantly high FAA content than those only treated with HPP. The clipfish treated with HPP at 400 MPa also had a significantly higher FAA content at the end of the experiment. Thus, SGS may cause formation of FAA. If the high level of FAA does cause off-flavor, the sensorial shelf life of the clip-fish may not reflect the microbial shelf life.

In conclusion, even though there was a synergistic effect between SGS and HPP in regard to microbial growth, it may have had the opposite effect in regard to FAA, which needs further investigation.

6 Future Perspectives

In the course of this work, certain subjects have surfaced that may be interesting to look at more closely:

- As the different packaging materials used in this study did not show any significant differences in microbial shelf life and the quality of the clip-fish, it would be interesting to perform similar experiments which aim to evaluate HPP and SGS impact on biodegradable packaging materials with lower OTR and WVTR than the biodegradable packaging material used.
- To enhance the arguments for replacing petroleum-based packaging materials with bio-based and biodegradable ones, it would be beneficial to conduct a more thorough examination, for instance, a life cycle analysis, that focused on how sustainable the production line of bio-based and biodegradable materials is.
- As the DSC analysis gave insufficient results, experiments that solely focused on how HPP affects protein denaturation could be of interest, for instance with different food products, and using different methods to evaluate the protein denaturation.
- Although the microbial shelf life of some of the treatment groups surpassed the experiment period of this study, the amount of FAA associated with flavor increased significantly. Thus, having a sensorial panel evaluating the food products smell and flavor would give a more accurate description of the clip-fish sensory shelf life.

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A Technical data sheet of cellulose film laminated to BioPBS

GROUNDED

13/11/2019

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

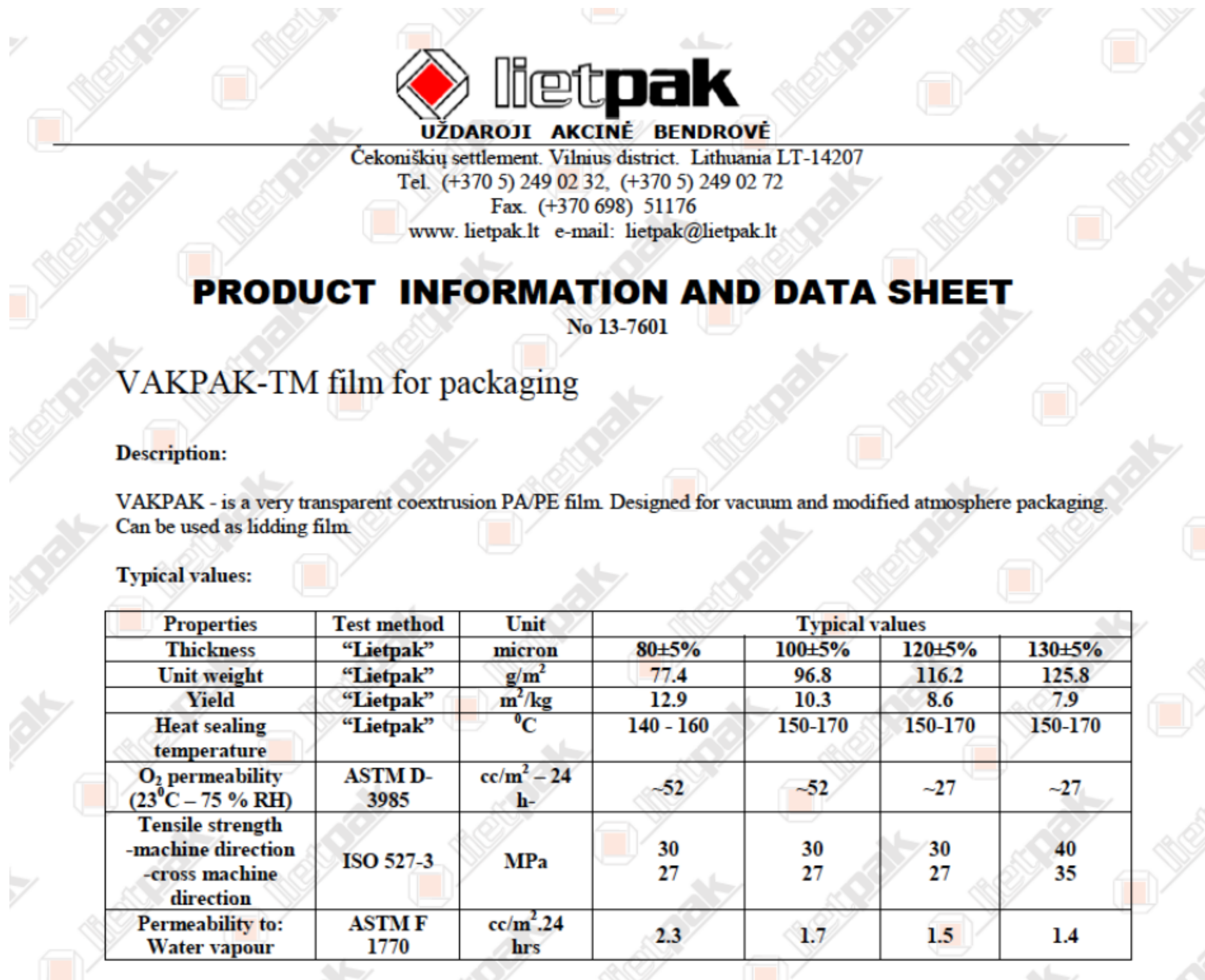
Product Type	Compostable Laminate	**Product Description**	Compostable Duplex Laminate
Specification Number	HCFD2 (b) (HK1)	**Product Structure**	19µ NK / Ink / Adhesive / 30µ Sealant Web
Production Method	Print / Laminate / Slit	**Approved Materials**	All materials conform to EN13432
Vincotte OK Compost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

Specification	**Tolerances**			
	Min	**Target**	**Max**	**Units**
Thickness	44.1	49	53.9	Micron
Yield	58.95	65.5	72.05	Gsm
COF (A/A Dynamic)	0.20	0.30	0.40	
WVTR 38°c | 90% RH (ASTM E96)		< 14		g / m² / 24hrs
OTR 23°c | 0% RH		< 1		cc / m² / 24hrs
Sealing Range (15psi 0.5sec dwell)	80	100	140	°C
Heat Sealing Strength		> 600		g / 25mm
Tensile Strength				
MD		71.46		N / mm²
TD		34.60		
Elongation To Break				
MD		16		%
TD		44		
Tear Strength				
MD		750		mN
TD		1350		

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 A.1: Technical data sheet of cellulose film laminated to BioPBS

B Technical data sheet of laminated multilateral PA/PE



lietpak

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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"	^0C	140 - 160	150-170	150-170	150-170
O_2 permeability (23^0C – 75 % RH)	ASTM D-3985	cc/m^2 – 24 h-	~52	~52	~27	~27
Tensile strength -machine direction -cross machine direction	ISO 527-3	MPa	30 27	30 27	30 27	40 35
Permeability to: Water vapour	ASTM F 1770	cc/m^2.24 hrs	2.3	1.7	1.5	1.4

Figure B.1: Technical data sheet of laminated multilateral PA/PE

C Dilutions for the microbial analysis

Table C.1: An overview of the dilutions used in the microbial analysis for each sample day.

	Replicate 1		Replicate 2	
Day	Iron Agar	L&H	Iron Agar	L&H
0	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	10^{-2}	10^{-2}	10^{-2}	10^{-2}
3	10^{-2}	10^{-2}	10^{-2}	10^{-1}
	10^{-4}	10^{-4}	10^{-3}	10^{-3}
			10^{-4}	10^{-4}
6	10^{-3}	10^{-3}	10^{-2}	10^{-2}
	10^{-4}	10^{-4}	10^{-4}	10^{-4}
	10^{-5}	10^{-5}	10^{-6}	10^{-6}
10	10^{-3}	10^{-3}	10^{-3}	10^{-3}
	10^{-5}	10^{-5}	10^{-5}	10^{-5}
13	10^{-1}	10^{-1}	10^{-1}	10^{-1}
	10^{-2}	10^{-2}	10^{-2}	10^{-2}
	10^{-3}	10^{-3}	10^{-3}	10^{-3}
	10^{-4}	10^{-4}	10^{-4}	10^{-4}
24	10^{-2}	10^{-2}	10^{-2}	10^{-2}
	10^{-4}	10^{-4}	10^{-4}	10^{-4}
	10^{-5}	10^{-5}	10^{-5}	10^{-5}
34	10^{-3}	10^{-3}	10^{-3}	10^{-3}
	10^{-5}	10^{-5}	10^{-5}	10^{-5}
48	10^{-4}	10^{-3}	10^{-4}	10^{-2}
	10^{-5}	10^{-5}	10^{-5}	10^{-4}

D Spesifications for Kjeldahl analysis

Table D.1: The specifications of the parameters used in Kjeldahl analysis.

Parameter	
H_2O volume	50 mL
NaOH volume	60 mL
Reaction time	5 s
Distillation mode	Fixed time
Distillation time	180 s
Stirrer speed distillation	10
Steam output	100%
Titration type	Boric acid (4%)
Receiving solution vol.	70 mL
Titration solution	H_2SO_4 0.25 mol
Sensor type	Colorimetric
Titration mode	Standard
Measuring mode	Endpoint pH
Endpoint pH	4.65
Stirrer speed titration	9
Titration strat volume	4 mL
Titration algorithm	Optimal

E Additional results

The following tables contain the means $\pm$ standard error of the mean for the measured values of total aerobic plate count and the total psychrotrophic count (Table E.1), the drip loss (Table E.2), and the textural measurements, firmness and braking force (Table E.3), and fillet hight (Table E.4) for all the samples throughout the experiment. The results from the Pearson correlation analysis are shown in Table E.6.

Table E.1: Means $\pm$ SEM of the total aerobic plate count (APC) and the total psychrotrophic count (TPC) measurement of all the treatment groups at each sample day (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively,

TAPC [log (cfu/g)]									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	$\mathbf{1.89 \pm 0.15}^{b}$	$\mathbf{1.57 \pm 0.09}^{a}$	1.62 ± 0.10^{a}	1.89 ± 0.20^{ab}	1.14 ± 0.11^{a}	1.82 ± 0.12^{ab}	1.72 ± 0.03^{ab}	1.75 ± 0.09^{ab}	$p_D < 0.001$
13	4.45 ± 0.37^{c}	3.87 ± 0.53^{c}	2.05 ± 0.12^{ab}	2.57 ± 0.35^{b}	2.12 ± 0.12^{ab}	2.31 ± 0.11^{ab}	2.36 ± 0.44^{ab}	1.81 ± 0.25^{a}	$p_{Pr} < 0.001$
24	6.59 ± 0.12^{b}	6.49 ± 0.10^{b}	3.47 ± 0.48^{a}	3.61 ± 0.40^{a}	3.40 ± 0.27^{a}	3.51 ± 0.39^{a}	3.28 ± 0.19^{a}	3.05 ± 0.12^{a}	$p_T < 0.001$
34			3.53 ± 0.14^{a}	3.90 ± 0.10^{a}	5.00 ± 0.35^{b}	5.15 ± 0.32^{b}	3.49 ± 0.30^{a}	4.06 ± 0.24^{a}	$p_{Pa} = 0.567$
48			5.30 ± 0.31^{a}	5.88 ± 0.04^{ab}	6.41 ± 0.10^{c}	6.09 ± 0.32^{bc}	4.48 ± 0.04^{a}	5.02 ± 0.14^{a}	

TPC [log (cfu/g)]									
Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	$\mathbf{2.31 \pm 0.00}^{a}$	$\mathbf{3.34 \pm 0.37}^{b}$	2.81 ± 0.50^{ab}	2.31 ± 0.00^{ab}	2.81 ± 0.00^{ab}	-	-	3.31 ± 0.00^{ab}	$p_D < 0.001$
13	4.78 ± 0.34^{b}	4.94 ± 0.37^{b}	2.31 ± 0.00^{a}	3.18 ± 0.30^{a}	-	-	3.05 ± 0.26^{a}	-	$p_{Pr} = 0.118$
24	6.85 ± 0.07^{d}	6.63 ± 0.08^{d}	4.55 ± 0.25^{bc}	5.19 ± 0.05^{c}	$\mathbf{3.98 \pm 0.39}^{ab}$	$\mathbf{5.00 \pm 0.17}^{c}$	3.81 ± 0.20^{ab}	3.31 ± 0.00^{a}	$p_T < 0.001$
34			4.31 ± 0.00	4.31 ± 0.00	5.15 ± 0.00	5.07 ± 0.28	-	4.79 ± 0.00	$p_{Pa} = 0.169$
48			$\mathbf{4.96 \pm 0.40}^{b}$	$\mathbf{5.97 \pm 0.10}^{cd}$	6.49 ± 0.16^{d}	6.10 ± 0.43^{d}	$\mathbf{4.26 \pm 0.22}^{a}$	$\mathbf{5.16 \pm 0.08}^{bc}$	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

- inserted where the dilution used was too high to notice any growth.

Table E.2: Mean ± SEM of the drip loss (DL) measurement of all the treatment groups at each sample day (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively,

Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	1.20 ± 0.15^{a}	1.50 ± 0.22^{a}	3.70 ± 0.48^{ab}	2.34 ± 0.60^{a}	2.10 ± 0.35^{a}	2.97 ± 0.81^{ab}	5.03 ± 1.26^{b}	3.81 ± 0.18^{ab}	$p_D < 0.001$
13	4.56 ± 1.22^{abcd}	2.34 ± 0.51^{ab}	**4.98 ± 1.30**^{bcd}	**2.05 ± 0.24**^{a}	5.62 ± 2.02^{cd}	4.03 ± 0.86^{abcd}	**3.84 ± 0.59**^{abc}	**6.55 ± 1.27**^{d}	$p_{Pr} = 0.280$
24	3.29 ± 0.85^{abc}	2.84 ± 0.47^{ab}	7.92 ± 0.61^{d}	7.73 ± 1.32^{d}	1.81 ± 0.27^{a}	2.47 ± 0.37^{ab}	4.59 ± 0.63^{bc}	5.72 ± 1.19^{cd}	$p_T < 0.001$
34			**5.11 ± 0.79**^{ab}	**8.41 ± 1.42**^{cd}	4.10 ± 0.72^{a}	3.99 ± 0.83^{a}	**7.41 ± 1.62**^{bc}	**10.45 ± 1.85**^{d}	$p_{Pa} = 0.289$
48			**6.02 ± 0.62**^{a}	**10.01 ± 0.46**^{b}	5.71 ± 1.28^{a}	4.48 ± 0.56^{a}	6.49 ± 0.77^{a}	6.69 ± 0.68^{a}	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

Table E.3: Mean of texture measurement (firmness, and braking force) of all the treatment groups at each sample day (n=6, in each treatment group). The fillet height has been used as a covariate. H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively,

Firmness [N]

Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	36.55 ± 3.52	35.88 ± 3.16	34.09 ± 3.18	36.11 ± 3.20	33.22 ± 3.16	33.60 ± 3.46	36.39 ± 3.16	28.46 ± 3.22	$p_D = 0.129$
13	35.35 ± 3.17^{ab}	35.19 ± 3.17^{ab}	37.25 ± 3.16^{ab}	42.09 ± 3.48^{b}	36.60 ± 3.58^{ab}	31.68 ± 3.46^{a}	33.10 ± 3.19^{ab}	31.85 ± 3.17^{a}	$p_{Pr} = 0.006$
24	**30.26 ± 3.16**^{a}	**42.40 ± 3.16**^{bc}	29.87 ± 3.23^{a}	28.07 ± 3.91^{a}	48.08 ± 3.16^{c}	45.77 ± 3.87^{bc}	40.74 ± 3.46^{bc}	36.23 ± 3.17^{ab}	$p_T = 0.002$
34			29.48 ± 3.16^{a}	30.55 ± 3.17^{ab}	44.85 ± 3.17^{c}	38.77 ± 3.17^{bc}	35.98 ± 3.50^{abc}	29.70 ± 3.16^{a}	$p_{Pa} = 0.194$
48			25.89 ± 3.46^{ab}	19.36 ± 3.17^{a}	42.91 ± 3.18^{d}	41.39 ± 3.16^{d}	38.99 ± 3.47^{cd}	31.72 ± 3.51^{bc}	

Braking force [N]

Treatment	H400-		SH400-		H600-		SH600-		
Day / Package	L	B	L	B	L	B	L	B	p-value[1]
0	40.47 ± 2.89	42.31 ± 2.87	40.59 ± 2.88	44.32 ± 2.90	41.75 ± 2.87	42.33 ± 3.14	43.89 ± 3.15	41.24 ± 2.92	$p_D = 0.092$
13	45.78 ± 3.16	40.57 ± 2.88	47.23 ± 3.51	46.60 ± 2.87	46.10 ± 3.25	41.77 ± 2.87	38.79 ± 2.90	39.94 ± 2.88	$p_{Pr} < 0.001$
24	**38.87 ± 2.87**^{a}	**52.34 ± 2.87**^{c}	34.72 ± 2.93^{a}	37.20 ± 3.55^{a}	51.87 ± 2.87^{c}	51.22 ± 3.52^{bc}	42.12 ± 3.14^{ab}	41.77 ± 2.88^{a}	$p_T < 0.001$
34			31.70 ± 2.88^{a}	37.34 ± 2.88^{ab}	**55.50 ± 3.16**^{d}	**45.36 ± 2.88**^{bc}	42.64 ± 3.18^{bc}	38.22 ± 2.87^{abc}	$p_{Pa} = 0.935$
48			26.74 ± 2.87^{a}	25.59 ± 2.88^{a}	54.18 ± 2.88^{c}	50.85 ± 2.87^{c}	39.07 ± 3.52^{b}	40.61 ± 2.91^{b}	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

Table E.4: Mean of the fillet hight [mm] measurement of all the treatment groups at each sample day (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package material, PA/PE and BioPBS, respectively,

	Treatment group							
Day	H400-L	H400-B	SH400-L	SH400-B	H600-L	H600-B	SH600-L	SH600-B
0	31.54 ± 2.62	30.13 ± 1.45	26.58 ± 1.61	32.74 ± 1.74	29.24 ± 1.25	28.70 ± 1.98	28.88 ± 2.17	33.56 ± 2.32
13	27.14 ± 1.90	30.76 ± 1.73	28.62 ± 1.83	30.59 ± 1.61	22.42 ± 1.64	29.52 ± 1.79	32.25 ± 1.58	27.20 ± 1.99
24	27.92 ± 2.47	27.77 ± 1.91	24.26 ± 2.27	24.80 ± 0.67	28.97 ± 1.05	30.47 ± 1.12	29.55 ± 0.81	27.11 ± 1.63
34			30.62 ± 1.27	26.85 ± 2.29	27.10 ± 1.61	30.99 ± 2.07	32.80 ± 1.01	29.17 ± 2.81
48			28.19 ± 1.57	31.31 ± 1.56	26.50 ± 1.72	30.32 ± 1.81	27.34 ± 0.59	33.06 ± 1.71

Table E.5: Mean measurement of the free amino acids distribution [mg/100g fish] amino acids not associated with flavor, of all the treatment groups from frozen samples taken on day 0, 24, and 48 (n=6, in each treatment group). H and SH denote processing with high-pressure processing, and soluble gas stabilization in addition to high-pressure processing, respectively, with either a pressure level of 400 or 600 MPa. L and B denote the different package materials, PA/PE and BioPBS, respectively,

Treatment		H400-		SH400-		H600-		SH600-		
Amino acid	Day	L	B	L	B	L	B	L	B	p-value[1]
Lys	0	5.43 ± 0.43	7.48 ± 0.42	6.12 ± 0.17	6.91 ± 0.71	6.53 ± 0.61	6.59 ± 0.83	6.43 ± 0.93	7.84 ± 0.46	$p_D = p_{Pr} = p_T < 0.001$
	24	14.86 ± 1.60^{a}	12.54 ± 1.05^{a}	38.76 ± 2.74^{b}	34.23 ± 5.39^{b}	10.73 ± 0.56^{a}	10.98 ± 0.73^{a}	33.83 ± 5.69^{b}	19.57 ± 1.14^{a}	$p_{Pa} = 0.652$
	48			85.24 ± 10.89^{c}	78.53 ± 14.09^{c}	14.26 ± 2.39^{a}	12.87 ± 1.15^{a}	31.82 ± 4.33^{b}	41.00 ± 6.50^{b}	
Asn	0	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.21 ± 0.04	0.19 ± 0.06	0.29 ± 0.07	0.39 ± 0.07	$p_D = p_{Pr} = p_T < 0.001$
	24	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.04 ± 0.01^{a}	0.03 ± 0.00^{a}	1.44 ± 0.30^{a}	1.11 ± 0.07^{a}	9.60 ± 1.48^{b}	8.05 ± 0.75^{b}	$p_{Pa} = 0.693$
	48			0.01 ± 0.00^{a}	0.03 ± 0.01^{a}	1.18 ± 0.54^{a}	0.79 ± 0.33^{a}	12.81 ± 3.38^{b}	13.33 ± 2.82^{b}	
Gln	0	2.37 ± 0.57	1.7 ± 40.19	1.99 ± 0.37	1.87 ± 0.24	2.05 ± 0.24	2.06 ± 0.28	2.09 ± 0.37	2.51 ± 0.31	$p_D = p_{Pr} = p_T < 0.001$
	24	6.72 ± 1.06^{a}	5.56 ± 0.51^{a}	15.54 ± 1.37^{b}	14.03 ± 2.47^{b}	4.53 ± 0.50^{a}	3.86 ± 0.43^{a}	$\mathbf{14.56 \pm 2.59}^{b}$	$\mathbf{8.36 \pm 1.23}^{a}$	$p_{Pa} = 0.676$
	48			28.05 ± 2.98^{c}	31.78 ± 6.52^{c}	5.15 ± 1.22^{a}	4.26 ± 0.16^{a}	13.31 ± 1.39^{b}	17.61 ± 2.78^{b}	
Tyr	0	1.65 ± 0.11	2.07 ± 0.18	1.77 ± 0.16	2.17 ± 0.27	1.81 ± 0.18	1.84 ± 0.24	1.81 ± 0.21	2.42 ± 0.28	$p_D = p_{Pr} = p_T < 0.001$
	24	4.57 ± 0.42^{a}	3.54 ± 0.29^{a}	11.59 ± 0.65^{c}	11.36 ± 1.79^{c}	3.58 ± 0.20^{a}	3.29 ± 0.25^{a}	9.27 ± 1.29^{bc}	6.15 ± 0.18^{ab}	$p_{Pa} = 0.732$
	48			24.81 ± 2.90^{c}	24.05 ± 4.08^{c}	4.53 ± 0.85^{a}	3.24 ± 0.25^{a}	9.66 ± 1.20^{b}	12.48 ± 1.62^{b}	

[1] The p-values was generated using GLM Univariate (Tests of Between-Subjects Effects, $p < 0.05$) where D = day, Pr = pressure, T = treatment, and Pa = packaging.

[abcd] Different letters indicate significant differences (Pairwise Comarisons GLM, $p < 0.05$) between treatment groups on the same day.

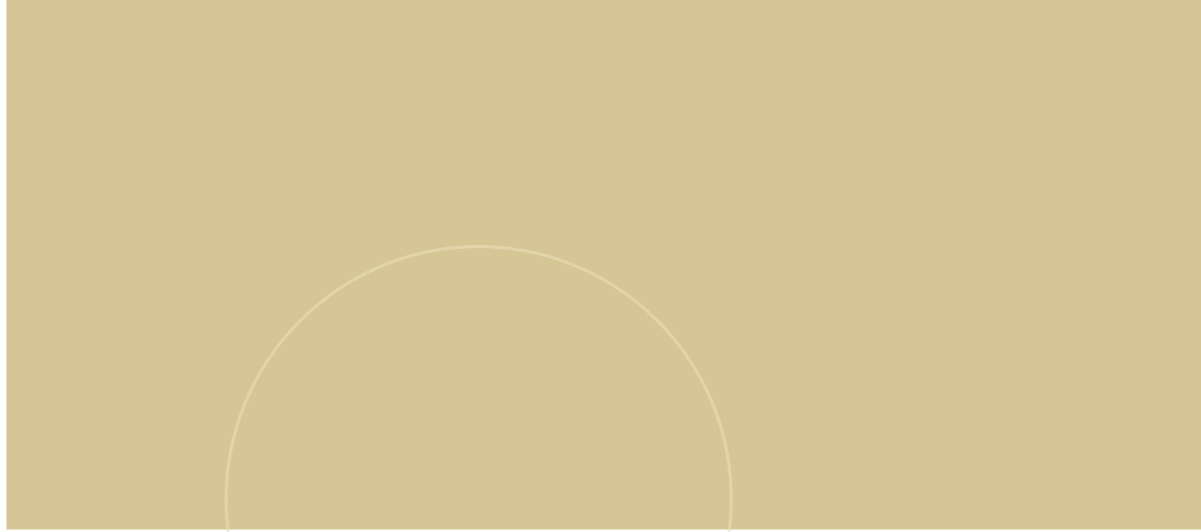
Bold text highlights significant differences ($p < 0.05$) pairwise from the same day, within the same treatment, with different packaging materials.

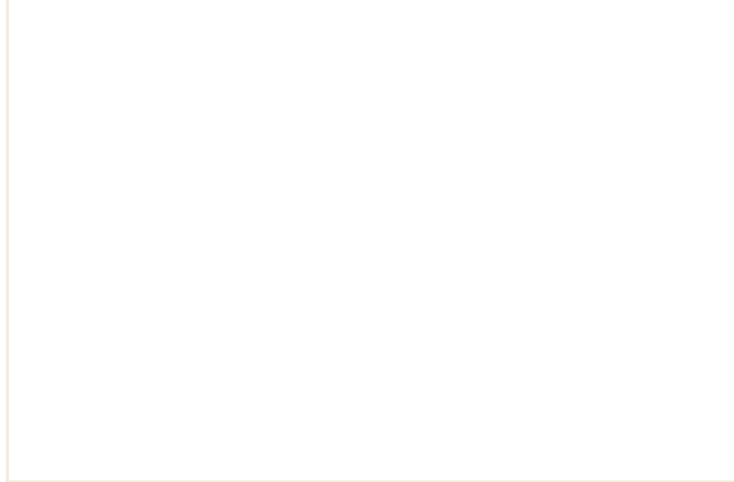
Table E.6: Persons Correlations of the quality parameters

Correlations																		
		drip loss [%]	water content [%]	protein content [%]	salt content [%]	fillethight [mm]	firmness [N]	breaking force [N]	pH	APC [log (cfu/g)]	TPC [log (cfu/g)]	Sweet FAA [mg/100g fish]	Bitter FAA [mg/100g fish]	Acidiy FAA [mg/100g fish]	L*	a*	b*	croma
water content [%]	Pearson Correlation	-0.065																
	Sig. (2-tailed)	0.375																
protein content [%]	Pearson Correlation	0.151	-.474**															
	Sig. (2-tailed)	0.100	0.000															
salt content [%]	Pearson Correlation	-.445**	-0.131	-0.030														
	Sig. (2-tailed)	0.005	0.403	0.844														
fillethight [mm]	Pearson Correlation	-.438**	-.310**	.176*	.614**													
	Sig. (2-tailed)	0.000	0.000	0.049	0.000													
firmness [N]	Pearson Correlation	-.603**	-.310**	.224*	.472**	.416**												
	Sig. (2-tailed)	0.000	0.000	0.012	0.002	0.000												
breaking force [N]	Pearson Correlation	-.324**	-.154*	0.065	0.113	0.057	.715**											
	Sig. (2-tailed)	0.000	0.035	0.470	0.476	0.425	0.000											
pH	Pearson Correlation	-.472**	.465**	-.200*	0.223	0.105	.179*	.170*										
	Sig. (2-tailed)	0.000	0.000	0.023	0.151	0.132	0.010	0.015										
APC [log (cfu/g)]	Pearson Correlation	.193**	-.235**	-0.063	0.168	-0.069	0.039	0.141	-.535**									
	Sig. (2-tailed)	0.009	0.002	0.495	0.287	0.349	0.600	0.056	0.000									
TPC [log (cfu/g)]	Pearson Correlation	0.210	-0.041	-.244*	-0.500	-0.092	-0.055	0.143	-.462**	.943**								
	Sig. (2-tailed)	0.051	0.712	0.041	0.117	0.392	0.610	0.176	0.000	0.000								
Sweet FAA [mg/100g fish]	Pearson Correlation	.433**	-.238**	0.107	0.248	-0.054	-.246**	-.391**	-.498**	.357**	0.059							
	Sig. (2-tailed)	0.000	0.008	0.228	0.104	0.550	0.005	0.000	0.000	0.000	0.623							
Bitter FAA [mg/100g fish]	Pearson Correlation	.553**	-.309**	.217*	0.177	-0.028	-.357**	-.514**	-.528**	.346**	0.074	.779**						
	Sig. (2-tailed)	0.000	0.000	0.014	0.252	0.752	0.000	0.000	0.000	0.000	0.534	0.000						
Acidiy FAA [mg/100g fish]	Pearson Correlation	.518**	-.268**	.199*	0.134	-0.029	-.345**	-.511**	-.525**	.376**	0.119	.771**	.973**					
	Sig. (2-tailed)	0.000	0.003	0.024	0.385	0.744	0.000	0.000	0.000	0.000	0.316	0.000	0.000					
L*	Pearson Correlation	-0.080	0.093	-0.062	-0.074	-0.080	-0.032	-0.059	0.090	-0.026	0.098	0.034	0.006	0.006				
	Sig. (2-tailed)	0.262	0.202	0.493	0.640	0.260	0.650	0.410	0.198	0.727	0.360	0.699	0.948	0.945				
a*	Pearson Correlation	-.150*	-.317**	0.175	0.284	.423**	.326**	0.114	-0.073	-0.105	-.267*	-.210*	-.192*	-.206*	-.535**			
	Sig. (2-tailed)	0.034	0.000	0.053	0.062	0.000	0.000	0.107	0.299	0.154	0.013	0.018	0.032	0.021	0.000			
b*	Pearson Correlation	0.075	-.259**	0.158	0.093	.165*	0.014	-0.031	-.280**	0.070	0.017	0.083	0.125	0.152	-.321**	.363**		
	Sig. (2-tailed)	0.291	0.000	0.079	0.559	0.019	0.844	0.661	0.000	0.345	0.874	0.351	0.159	0.086	0.000	0.000		
croma	Pearson Correlation	0.076	-.258**	0.155	0.093	.164*	0.012	-0.033	-.284**	0.075	0.024	0.083	0.126	0.153	-.315**	.361**	1.000**	
	Sig. (2-tailed)	0.285	0.000	0.084	0.560	0.020	0.865	0.647	0.000	0.306	0.821	0.350	0.157	0.084	0.000	0.000	0.000	
hue	Pearson Correlation	-0.066	-.144*	0.090	0.016	.181*	0.121	-0.010	0.102	-.219**	-.213*	-.240**	-0.116	-0.127	-.369**	.540**	.291**	.283**
	Sig. (2-tailed)	0.359	0.049	0.322	0.919	0.011	0.090	0.894	0.149	0.003	0.049	0.007	0.198	0.160	0.000	0.000	0.000	0.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).





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