Master's thesis

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Application of soluble gas stabilization (SGS) on pre- and post-rigor filleted salmon prior to packaging

Master's thesis in Chemical Engineering and Biotechnology
Supervisor: Anita Nordeng Jakobsen
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Abstract

The aim of the thesis was to investigate whether saturation of Atlantic salmon with CO_2 prior to packaging using soluble gas stabilization (SGS) technology enhances storage quality. The method was conducted on pre-rigor and post-rigor filleted salmon to observe if time of filleting affects the effect of SGS-processing.

Carbon dioxide is extensively used in food packaging as it has an inhibiting effect on microbial growth and has shown a favorable impact on shelf-life. Carbon dioxide is one of the primary gases in modified atmosphere packaging (MAP) and the key contributor to preventing spoilage. However, the inhibiting effect has limitations, which might be associated with insufficient amounts of CO_2. There exist limited amounts of literature on SGS, and it is still unclear whether SGS can be combined with vacuum packaging, or if rigor mortis affects the effect of SGS. This study extends prior work by investigating quality aspects of both pre- and post-rigor filleted salmon stored in MAP and vacuum where soluble gas technology has been applied.

The effect of SGS prior to packaging on storage quality was investigated by determining the concentration of ATP degradation products, K- and H-value, the total aerobic plate counts (APC), H_2S-producing bacteria counts, and evaluate the color characteristics lightness (L*), redness (a*) and yellowness (b*). The results of the study indicated that SGS was most beneficial in terms of spoilage and not freshness. H-values of SGS treated post-rigor filleted samples were significantly lower than the control ($p=0.004$), whereas SGS had no apparent advantage on K-values. In brief, SGS-processing seemed to restrain the microbial growth and enzyme activity, meanwhile, the endogenous enzyme activity appeared unaffected.

This study suggests that SGS is a promising approach to increase shelf-life, especially when combined with vacuum packaging. Post-rigor SGS-vacuum samples had significantly lower amounts of hypoxanthine than conventional vacuum ($p=0.002$) and MAP samples ($p=0.038$). Furthermore, the amount of inosine in post-rigor SGS-vacuum samples was significantly higher ($p<0.05$) than post-rigor vacuum, MAP and SGS-MAP samples. The advantage of SGS was more evident when combined with vacuum than MAP. Except for pre-rigor SGS-MAP samples having an increased fillet redness (a*) compared to the control ($p=0.024$), SGS had no significant impact on the color characteristics throughout storage. Moreover, pre- and post-rigor SGS-vacuum samples had the overall lowest APC and H_2S-producing bacteria counts, though not significantly. Finally, early filleting has been suggested as an adequate filleting approach when combined with SGS.

The future potential of SGS is to reduce the degree of filling (DF) of MAP packages and/or use SGS-vacuum in preference to conventional MAP, thus increasing transport efficiency.

Keywords: SGS-technology, food packaging technique, preservation, pre-and post-rigor, shelf-life

Sammendrag

Målet med masteren var å undersøke om innløsning av CO_2 før pakking, ved bruk av soluble-gas-stabilization (SGS) teknologi, forbedrer lagringskvaliteten til laks. Metoden ble utført på pre-rigor og post-rigor filetert laks for å se om tidspunkt av filetering hadde innvirkning på effekten av SGS.

Ettersom CO_2 har en hemmende effekt på mikrobiell vekst, samt en gunstige effekter på holdbarhet, er gassen ofte blitt benyttet i matemballasje. Karbondioksid er en av de viktigste gassene i modifisert atmosfære pakking (MAP) og den viktigste bidragsyteren til å forhindre forringelse. Likevel er effekten begrenset, noe som kan være knyttet til utilstrekkelige mengder CO_2. Det finnes begrenset med litteratur på SGS og det er blant annet uvisst om SGS kan kombineres med vakuumemballasje, samt om rigor mortis har en påvirkning på effekten. Denne studien bygger videre på tidligere arbeid ved å undersøke kvalitetsaspekter til både pre- og post-rigor filetert SGS behandlet laks lagret i MAP og vakuum.

Effekten av SGS på ulike kvalitetsaspekter ble undersøkt ved å bestemme konsentrasjonen av ATP-nedbrytningsprodukter, K- og H-verdi, totale aerobe kimtall (APC), H_2S-produserende bakterie kimtall, samt evaluere fargeegenskapene lyshet (L*), rødhet (a*) og gulhet (b*). Resultatene fra studien indikerte at SGS var mest fordelaktig i å utsette forringelse, ikke bevare ferskhet. H-verdien til SGS-behandlede post-rigor fileterte prøver var signifikant lavere enn kontrollprøven ($p = 0.004$), samtidig som SGS ikke hadde noe tilsynelatende effekt på K-verdiene. SGS-prosessering så ut til å begrense enzym aktiviteten, samt den mikrobielle veksten i vakumerte prøver, mens aktiviteten til de endogene enzymene virket upåvirket.

Studien antyder at SGS er en lovende tilnærming for å øke holdbarheten, spesielt i kombinasjon med vakuum. Post-rigor filetert SGS-vakuum prøver hadde signifikant lavere konsentrasjon av hypoxanthine enn konvensjonelle vakuum ($p=0.002$) og MAP prøver ($p=0.038$). Videre var mengden inosin i post-rigor SGS-vakuum prøver signifikant høyere ($p<0.05$) enn post-rigor vakuum, MAP- og SGS-MAP-prøvene. Fordelen med SGS var betydelig større i kombinasjon med vakuum enn med MAP. Med unntak av pre-rigor SGS-MAP prøver som hadde en økt filet rødhet (a*) ($p=0.024$), hadde SGS ingen signifikant innvirkninger på fargekarakteristikken under lagring. Dessuten hadde pre- og post-rigor SGS-vakuum prøver generelt de laveste APC- og H_2S-produserende bakterie kimtallene, dog ikke signifikant. Til slutt har pre-rigor filetering i kombinasjon med SGS blitt foreslått som et egnet fileteringstidspunkt.

Et fremtidige potensial ved bruk av SGS er å redusere fyllingsgraden (DF) av MAP-pakker og/eller benytte SGS-vakuum i stedet for MAP, og dermed øke transportseffektiviteten.

Nøkkelord: SGS-teknologi, emballasjeteknologi, konservering, pre- og post-rigor filletering, holdbarhet

Acknowledgement

This thesis was conducted at the Department of Biotechnology and Food Science at the Norwegian University of Science and Technology (NTNU) in Trondheim. The work is part of the project 'SGS-Concept' and is the final assignment of the Chemical Engineering and Biotechnology study program, corresponding to 30 ECTS.

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Furthermore, I would like to thank my fellow students who have been of much support over the past five years. All hours together, both on- and off-campus, have been greatly appreciated. Lastly, I want to thank my family for all encouragement and a big shout out to my current flatmates whom I have lived with more or less the entire studying time. It has been a blast with many great memories to look back on.

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Abbreviations

SGS	=	Soluble Gas Stabilization
In	=	Inosine
Hx	=	Hypoxanthine
IA	=	Iron Agar
APC	=	Aerobic plate count
SSO	=	Specific spoilage bacteria
HPLC	=	High-performance liquid chromatography
SD	=	Standard deviation
SE	=	Standard error
L*	=	CIE lightness parameter
a*	=	CIE red (+)/green (-) parameter
b*	=	CIE yellow (+) / blue (-) parameter

Chapter 1

Introduction

Norwegian salmon is a highly successful product and has become a well-known trademark on a global basis. In 2019, the export of seafood in Norway made history as it exceeded a value of 100 billion NOK and the Norwegian seafood council announced that salmon contributed to 72.5 billion, making salmon the most profitable seafood product [1]. Furthermore, the Norwegian seafood council asserts that on a global scale, 50 percent of the produced salmon originate from Norway. It is clear that Norway has a major market share in the global marine market. Thus, it is important to further develop the Norwegian industry to retain the leading position in the market [2].

Over the last years, sustainability and the environment has become highly debated topics. Thus, food production must be compatible with a sustainable future. The administrative director of the Norwegian seafood council, Renate Larsen, states that the Norwegian salmon is considered safe, sustainable and of high quality. Larsen further suggests that the export value can be as much as doubled over the next ten years to 200 billion NOK, given that Norwegian salmon is still deemed as the most sustainable choice [1]. This emphasizes the relevance of new technologies, which contributes towards greener development, and as a result, making Norwegian salmon and salmon products even more attractive.

Food waste is a global threat against sustainable development and the increasing population. In 2015, the United Nations proposed seventeen sustainable development goals to be achieved by 2030. The second goal involves eradicating hunger by investing in sustainable food production and productivity [3]. When it comes to fish, a considerable amount of the captured fish never reach the consumers' table. The Food and Agriculture Organization of the United Nations (FAO) reported in 2015 that post-harvest losses accounted for 10 % of the global capture and cultured fish. FAO stated that many factors affect the shelf-life and quality of the fish product, such as post-harvest handling, processing, preservation, packaging, and storage [4]. However, compared to many other food products, fish can easily spoil as specific spoilage microorganisms (SSO) grow well even in chilled environments [5].

A novel approach to prolong the shelf-life and/or reduce the environmental impact is Soluble gas stabilization (SGS) technology. Soluble gas stabilization utilizes the preservative effect of CO_2 by

saturating the product with CO_2 prior to packaging. The carbon dioxide dissolves into the product and serves as an antimicrobial agent. The technique has shown promising results in increasing the shelf-life and thus contributes to decreasing the prevalent food waste problem and contribute towards a sustainable development. Furthermore, SGS has a potential in increasing the degree of filling (DF) without compromising the bacteriostatic effect and without having challenges associated with snug down. An increased DF is important in the matter of climate emissions related to logistics as more product can be transported per volume, as well as reduce the amount of required packaging. Soluble gas stabilization technology is therefore highly relevant in terms of economic benefits as well as satisfying the increasing demand for sustainable food.

Norwegian salmon can become even more profitable if products with better properties are developed. Today, the export of salmon is mainly gutted or filleted fish [6]. There exist an unexplored potential regarding product development of Atlantic salmon, which in the long run, can increase the demand even further. The Norwegian Seafood Council claims that in order to further increase the value of Norwegian fish export, the industry should focus on increasing the amount of processed fish. According to the Norwegian Seafood Council, the industry is missing out on billions as the majority of Norwegian salmon is exported without being processed [7]. New packaging technology, such as SGS, can contribute to a unique product that can result in reaching new, potential markets.

Previous research on soluble gas stabilization has shown promising results. From a microbiological perspective, SGS-treatment of Ready-to-Eat shrimps resulted in a significant reduction in the aerobic plate count (APC) and psychrotrophic count (PC) ($p<0.01$). Moreover, the application of SGS significantly increased sensory quality ($p<0.01$) [8]. When applied to Atlantic Halibut, Rotabakk *et al.* demonstrated a significant effect ($p<0.001$) of the SGS treatment on the CO_2 content in packages, which counteracted package collapse. Furthermore, APC and PC was also significantly reduced ($p=0.038$). Additionally, SGS treatment significantly reduced the drip loss ($p=0.006$) [9]. Similar results have been reported for skinless chicken breast fillets as well. APC was significantly ($P<0.015$) decreased in SGS treated products [10]. Even though SGS-technology reduces APC, not all aerobic microorganisms are spoilage bacteria. However, there is a better chance of having more spoilage bacteria in samples with high aerobic plate count than samples with lower counts. A high number of spoilage microorganisms results in a considerable shortening of the shelf-life. Despite the knowledge of the inhibiting effect of CO_2, the amount of literature concerning the application of SGS is limited.

Aims and objectives

Established knowledge has clearly demonstrated the inhibiting effect of CO_2 on microbial growth, and how saturation with CO_2 prior to MAP enhance quality aspects [8–10]. However, previous research has not addressed the effect of combining SGS-technology with vacuum packaging. Furthermore, less attention has been paid to rigor mortis and if it affects the SGS-treated products, i.e. if time of filleting prior to solubilization has an impact on quality and/or shelf-life. Thus, this thesis set out to investigate whether SGS-processing of pre-rigor filleted salmon enhances the storage quality and/or achieve a prolonged shelf-life compared to post-rigor filleted SGS-treated salmon.

To summarize the aims, the present research tries to

- determine whether SGS-processing enhance the quality or inhibit autolytic changes and microbial spoilage by comparing SGS-vacuum and SGS-MAP stored salmon with conventional packaging.
- determine if time of filleting alters the quality of SGS processed products stored in MAP and vacuum by comparing pre- and post-rigor filleted, SGS processed salmon.
- determine the impact of storage time on quality and shelf-life of SGS-processed products compared to conventional by monitoring parameters over a given period.

Based on the project report proceeding the thesis, the following parameters were chosen to investigate the quality and spoilage aspects; the total aerobic plate count, H_2S-producing bacteria count, amount of headspace CO_2, pH, amount of ATP degradation compounds and colorimetric properties. Food safety has not been involved in this study.

Extending previous work

As the project report proceeding this thesis investigated the effect of SGS-processing on pre-rigor filleted salmon, this thesis extends prior work by including post-rigor filleted salmon to determine if time of filleting affect the SGS-treatment. Moreover, the project report included quantification of the spoilage organism *Brochothrix thermosphacta*, which was only sporadically detected, as well as the lactic acid bacteria count and total aerobic plate count (APC), which were low. Earlier findings by Rotabakk et al. (2008) also reported *Brochothrix thermosphacta* to be unaffected by SGS-processing [9]. Hence, only APC and H_2S-producing bacteria count were chosen to evaluate the microbial growth in this thesis.

Even though *Photobacterium phosphoreum* is one of the main spoilage bacteria in modified atmosphere packed products, the growth was not investigated in the previous project report. In the present thesis, it was decided to focus on the autolytical processes, hence determining the count of *Photobacterium phosphoreum* was not given priority. In retrospect, this should have been investigated as APC is not always sufficient when explaining trends, for instance during the later stages of ATP degradation. Texture analysis was also carried out in the previous report. However, the results were not valid as the samples were not able to withhold its position while the cylinder probe applied pressure, hence texture was not further investigated.

Challenges

Due to covid-19 and restrictions in the laboratory access, several analyses were not finalized. Among the task were determination of biogenic amines, identification of the microbiological community by sequencing 16S rRNA genes from the total DNA extracted from the samples, and quantify the amount of solubilized CO_2 in MA-packages. Furthermore, exceptionally low atmospheric pressure during storage caused problems for the MA-packages. First, when measuring the buoyancy, in order to determine the solubility of CO_2, the low atmospheric pressure disrupted the equilibrium, causing incorrect measurements. Second, it may have influenced the results as varying amounts of CO_2 diffused out of the tissue during storage, causing package bulging of different magnitudes. Thus, groups of the same processing and storage had individual differences regarding the amount of dissolved CO_2. This may explain some of the individual deviations.

Moreover, there were challenges related to working with biological material. As large individual variations occurred in the stored salmon, *n* should have been greater in order to obtain more accurate data. In an attempt to compensate for individual differences, the samples were randomized. On the contrary, *n* equal to three is most likely not sufficient to provide a representative selection. However, a larger *n* would not have been practically feasible.

Key terms used in the thesis

- The phrase 'groups/samples of equal processing and packaging' will be used in this thesis to distinguish between the four main groups; samples stored in vacuum, SGS-vacuum, MAP and SGS-MAP.
- The term 'time of filleting' will be used in this thesis to differentiate between the two chosen filleting approaches; pre-rigor and post-rigor filleting

Highlights

- The advantage of SGS is more evident between vacuum and SGS-vacuum samples, than between MAP and SGS-MAP samples.
- Vacuum packed salmon stand out negatively compared to all investigated storage conditions.
- SGS seems to inhibit the activity of microbial enzymes responsible for converting In into Hx, whereas the activity of endogenous enzymes appears unaffected.
- Although the advantage of SGS is more evident in post-rigor filleted, pre-rigor filleting is suited for SGS-processing.
- Headspace gas composition was unaffected by time of filleting. Further research should be conducted to determine if the amount of solubilized CO_2 is influenced by the time of filleting.

- The SGS-processing of MAP and vacuum packed salmon did not have any significant effect on the fillet lightness (L*), redness (a*), and yellowness (b*). Exception: Pre-rigor SGS-MAP samples had significantly higher fillet redness (a*) than the control. However, the significance of these results on quality is assumed marginal.

Chapter 2

Literature review

2.1 Post-mortem changes in fish

Subsequently to death, the cells in the muscle tissue will not be supplied with oxygen as the blood stops to circulate. In living fish, glycogen is degraded to ATP, carbon dioxide and water through aerobic glycolysis. However, when slaughtered, the fish muscle becomes anaerobic and the glycogen is degraded through anaerobic glycolysis where the major end product is lactic acid [11]. As a result, the lactic acid accumulates and the pH decreases depending on the amount of glycogen converted into lactic acid, which in turn is dependent on the amount of stored carbohydrate (glycogen deposit) in the living tissue [12]. A lowering in pH is favorable as the fish muscle becomes less susceptible to microbial attack [11]. Thus, high glycogen deposits are beneficial as more glycogen can be converted into lactic acid. Accordingly, stressed fish prior to slaughter should be avoided as their glycogen deposits are depleted and will not achieve a pH-drop as high as for unstressed fish.

Post-mortem changes of fish are often classified into two categories: autolytic changes and bacterial spoilage. At first, major changes occur due to autolysis (*auto*-self, *lysis*-splitting, i.e. self-digestion) carried out by endogenous enzymes (*endo*-in, *genís*-create, i.e. inherent) [13]. These enzymes degrade ATP into smaller components, which are associated with freshness, its depletion, and spoilage.

Accordingly, these metabolites can be monitored and used as indices of freshness and spoilage [14]. At first, endogenous enzymes are mainly responsible for the ATP degradation. However, during storage, the bacterial enzyme activity will evolve and become primarily responsible for major changes occurring in the fish, as illustrated in Figure 2.1. These changes, caused by the microbial enzyme activity, are linked to spoilage [13]. Figure 2.1 illustrate the quality changes in cod and the main contributor to the changes throughout storage. Phase 1 and 2 are associated with freshness and its depletion mainly caused by endogenous enzymes, whereas phase 3 and 4 involve spoilage development caused by microbial enzymes [12]. The sequence of actions applies to salmon as well, though at different magnitudes.

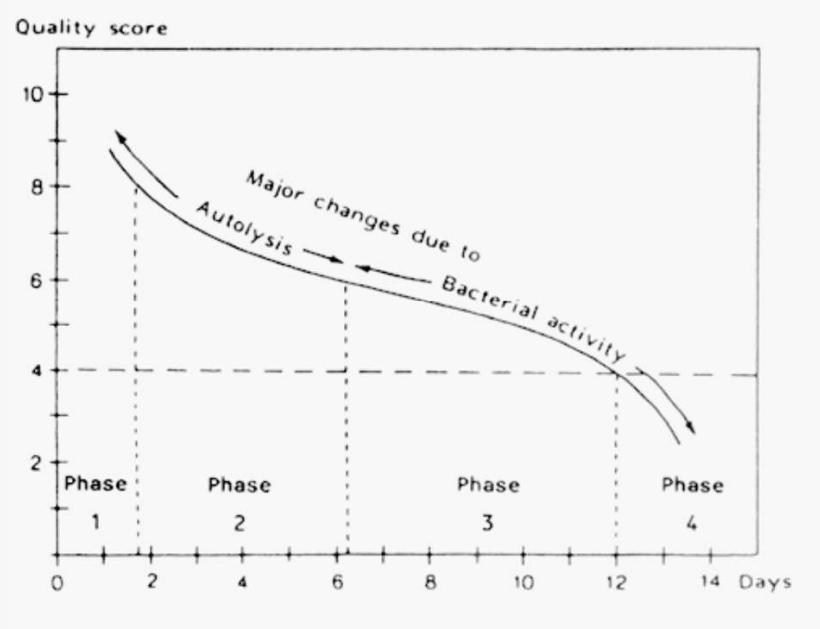


Figure 2.1: Different phases of changes occurring in cod during storage. The illustration is collected from[15].

2.1.1 Autolytic changes

Rigor mortis

Subsequently to death, the fish muscle enters a phase of rigor, which is recognized by the stiffening of muscle tissue. It is a result of muscles remaining in a locked and inflexible position. The fish muscle is referred to as pre-rigor before death-stiffening occur and post-rigor when it has ceased off [16]. Shortly after slaughter, the muscle is subjected to autolytic changes resulting in the conversion of muscle into meat [17]. The biochemical events related to this transition will be presented in the following sections.

Before death, muscles operate by muscle contractions and relaxations. This mechanism is caused by the interaction between actin and myosin filaments. These proteins are organized in an alternate pattern and form a unit called sarcomere as illustrated in Figure 2.2 (a). The contraction starts when the energy-carrying molecule ATP binds to myosin illustrated in Figure 2.2 (c). The energy causes myosin heads to attach to actin and this action is regulated by the presence of Ca^{2+}ions which exposes the myosin-binding site on the actin filament, see Figure 2.2 (b). Myosin utilizes the energy from ATP to pull the actin towards the M-line, which is the attachment site of the myosin filaments. This action is often referred to as the power stroke and results in a contracted sarcomere with overlapping filaments, illustrated in Figure 2.2(a). Myosin remains locked to actin until another ATP molecule binds and releases it from actin. After the unattachment, the muscle can either go through another cycle of binding, causing further contraction, or remain unattached, allowing the muscle to stretch [17].

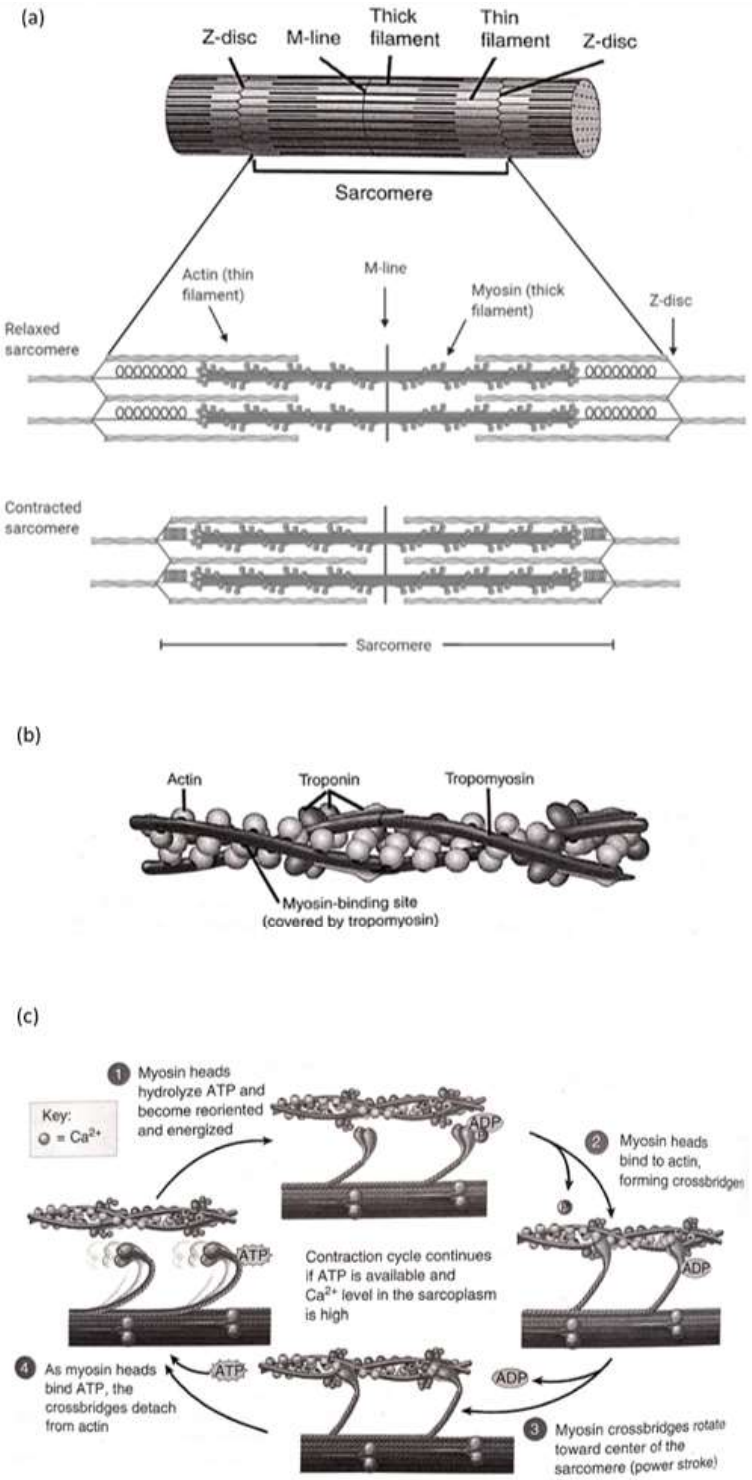


Figure 2.2: Illustration of the muscle structure. (a) The myofibril structure consisting of subsequent units of sarcomeres. (b) The structural arrangement of the actin (thin) filament. (c) The muscle contraction cycle which involves interaction between actin- and myosin filaments. Myosin attaches to actin and pulls it towards the M-line. (a), (b), and (c) has been modified from Tortora, G.J. and B. Derrickson (2006). *Principles of anatomy and physiology*. John wiley and sons, Inc., Hoboken, NJ. using Biorender.com.

The phase of rigor occurs when the intracellular level of ATP is not sufficient to unlock myosin from actin filaments in the muscle. Thus, the muscle will remain in the contracted position and appear stiff [16]. The level of ATP in the muscle of living fish is typically 7-8 moles/g, while the level in muscle entering rigor has decreased to 1.0 moles/g. The decrease is a result of the limited ATP production by the anaerobic glycolysis. Usually, when ATP is produced through aerobic glycolysis, 36 moles of ATP are produced per mole of glucose. However, as post-mortem generation of ATP, however, takes place in anaerobic tissue, the anaerobic glycolysis pathway is utilized, and only 2 moles of ATP are synthesized per mole of glucose. As a result, the level of ATP in aerobic fish muscle cannot be maintained in the anaerobic fish muscle, and eventually, it will become stiff [12].

Church (1998) reported that post-mortem metabolism in fish causes a pH drop from 7.4 to around 6, [18] while others reports a final postmortem pH of 6.5-6.7 [19]. A reduced pH is favorable in terms of bacteriostatic effect. However, it also causes a higher drip loss as the water-holding capacity decreases. Myosin, which is the most dominant muscle protein, has an isoelectric point close to pH 5.0. Accordingly, when the postmortem pH approaches the isoelectric point, the protein-protein interactions will increase and the protein-water interactions will decrease. As a result, water is released from the tissue. This is undesirable from an economic point of view as fish is often sold by weight and may be perceived by the consumer as less juicy and tender [17]. Furthermore, acidic environments also affect color, giving a paler appearance. However, it is important to emphasize that the postmortem pH level of fish is high compared to other species. In beef, for instance, the high glycogen levels in the muscles result in more lactic acid accumulation and a pH drop to around 5.5-5.6 [20].

Autolytic changes involving proteolytic enzymes

During the resolution of rigor mortis, the muscular tissue begins to soften. As ATP is absent, the actin-myosin complex is still in a locked position. Thus, softening and flexibility of the tissue occur due to post mortem proteolysis and not the presence of ATP. Proteolysis of myofibrillar tissue, i.e. myofilament framework, and connective tissue causes structural deterioration of the muscular tissue. The structural and biochemical changes through rigor are mainly caused by the action of endogenous proteolytic enzymes [17, 19] When pH and the intramuscular ionic strength changes in post-mortem muscle, it may activate or inhibit the activity of proteases. Furthermore, it causes alterations of the myofibrillar conformation, thus increasing the susceptibility to proteolytic cleavage [21].

Examples of some proteolytic enzymes which are believed to influence the post mortem proteolysis in salmon are matrix metalloproteinases [22], cathepsins [23, 24], and calpains [24]. Cathepsins are acid proteases that are usually inactive in living tissue. They are located in the lysosomes and released into the cytosol post mortem due to the muscle acidification which disrupts the lysosomal membrane [25]. The activity of cathepsin B+L and B in pre-rigor ice stored fillets of Atlantic salmon was found by Gaarder *et al.* (2012) to increase significantly after filleting [24]. Furthermore, Bahuaud *et al.* (2010) reported a significant negative correlation between fillet firmness and level of cathepsin L activity in Atlantic salmon [26]. Additionally, cathepsin D was shown by Yamashita and Konagaya (1990) to degrade myofibrillar proteins (actomyosin)

and connective tissue [27]. Calpains are cysteine proteases and are most active at neutral pH (6.9-7.5) [28]. Their action in post-mortem degradation is believed to be cleavage of myofibrillar proteins [28–30]. They are dependent on calcium, however, the regulation of its activity is not fully understood. Lastly, matrix metalloproteinases, catalysing the connective tissue catabolism, are zinc and calcium-dependent. They cause degradation of collagen and cytoskeletal proteins, which connect the sarcolemma to the extracellular matrix [31].

Membrane function and permeability

Ante mortem fish has defense mechanisms and barriers in order to prevent microorganisms from penetrating the fish tissue. However, under post mortem conditions the defences, for instance, the immune system [12], are disabled, making the mechanical barriers in the skin and membranes along the intestinal wall more permeable. Consequently, the fish becomes more susceptible to microbial growth [32]. However, only a limited amount of bacteria was reported by Murray and Shewan (1979) to invade the flesh during iced storage [33]. It was proposed by Huss (1995) that since the microbial growth mainly occurs at the surface, microbial spoilage is most likely a consequence of bacterial enzyme diffusion through the flesh and nutrient diffusion to the outside [12]. The autolysis also causes degradation of components which makes it easier for microorganisms to digest them.

Post-mortem degradation of nucleotides

Post-mortem ATP degradation in fish muscle is highly correlated to freshness, and autolytic changes are the main cause of the initial quality loss [13]. When nucleotides are degraded, it affects the taste, odour and the overall quality of the fish. The high energetic nucleotide ATP is degraded in several steps into Hypoxanthin (Hx). However, the last steps are mainly catalysed by microbial enzymes and linked to microbial spoilage. Even though the degradation of ATP by endogenous enzymes are not causing spoilage directly, it contributes by making catabolites available for microbial growth, thus increasing the microbially induced degradation processes. [34] A schematic explanation of the nucleotide degradation from ATP to Hx is given in Figure 2.3.

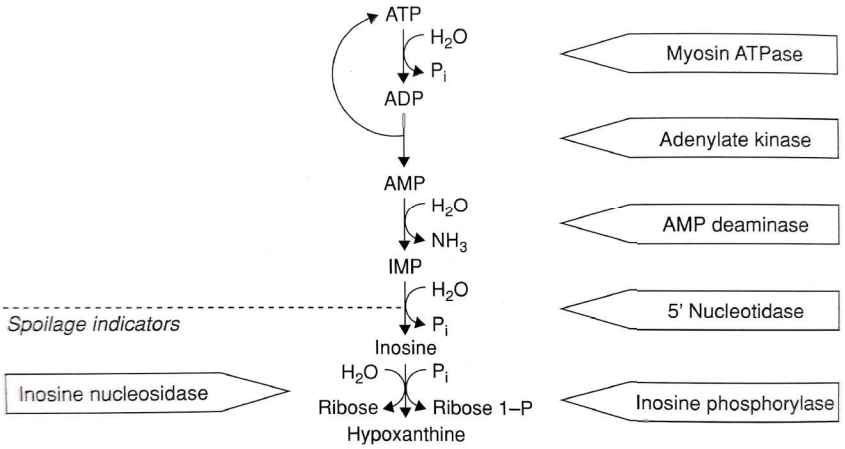


Figure 2.3: Overview of the postmortem nucleotide degradation pathway from ATP to hypoxanthine. P_i represent inorganic phosphate and the enzymes, catalysing the different steps, are indicated in the arrows. The dotted line represent the beginning of spoilage indicator substances, mainly formed by microbial enzymes. The figure is collected from [19].

With the intention of placing post mortem nucleotide degradation in relation to post mortem changes, a simplified overview illustrating the order of events following capture is given in Figure 2.4.

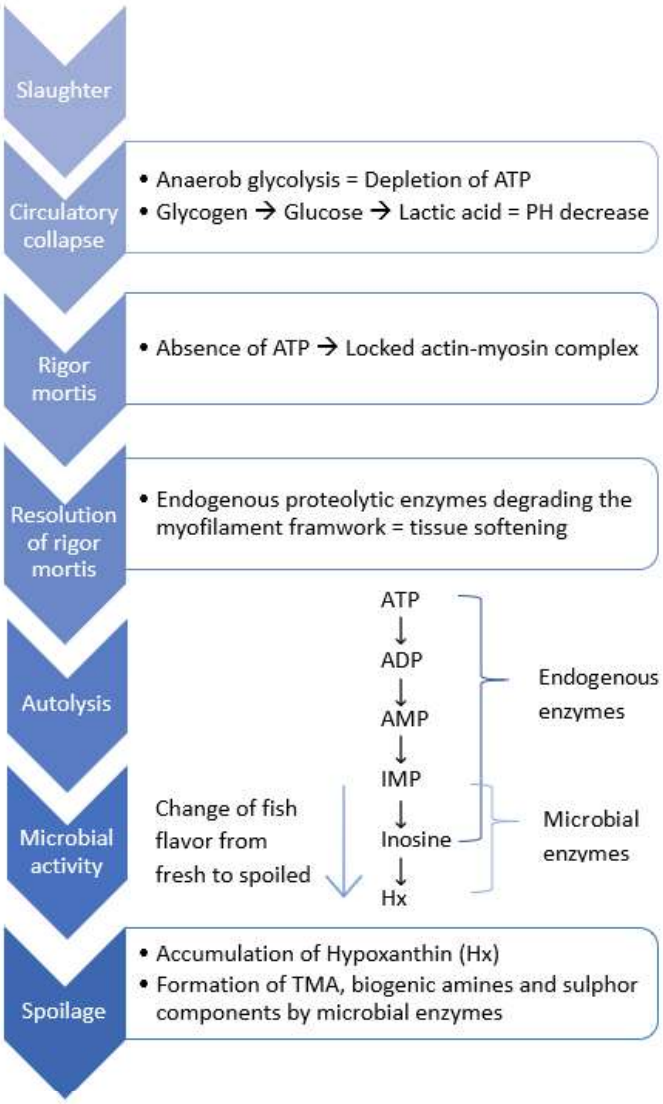


Figure 2.4: The sequence of events following capture. The overview was redrawn based figures from [31] and [35].

The degradation of ATP to Inosine (In) is mainly caused by the activity of endogenous enzymes. The formation of In is considered to be the last, autolytic step of the nucleotide catabolism [19]. Thus, the formation of In is regarded as an indication of a decline in the superior quality of seafood [36–38]. As the microbial growth accelerates, the microorganisms contribute to further degradation of In into Hx. Although Hx has been found to accumulate in sterile fish tissue, indicating conversion due to endogenous enzyme activity, this process has been notably slow. Hence, microbial enzymes are the main contributors when degrading In[34].

Various types of ATPase can degrade ATP, where Ca^{2+}-dependent myosin ATPase is the most common. This enzyme is directly involved in muscle contraction, thus when $Ca^{2+}$is released in the sarcoplasmic reticulum (SR), the rate of ATP degradation is increased tremendously. Stressed

fish have high amounts of Ca^{2+} present in SR, due to the high energy need when moving vigorously. Consequently, the myosin ATPase activity is high in stressed fish, resulting in high consumption of ATP also after slaughtered. If the ATP depletion is rapid, as in the case of stressed fish, a strong form of rigor mortis follows, referred to as thaw rigor [19].

High levels of the intermediate nucleotide IMP is associated with the umami taste, thus giving a pleasant taste. Conversely, fish is perceived as less fresh when the IMP level ceases [39]. As ATP degradation is closely related to freshness, a freshness indicator has been developed. It is referred to as K-value and is based on the concentrations of the ATP metabolites in the following manner, [40]

$$K - value(\%) = \frac{[In] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [In] + [Hx]} \cdot 100 \tag{2.1}$$

The K-value is referred to by Hong *et al.* (2017) to be one of the most useful indicators of fish freshness [39]. Erikson and Misimi (1997) outlined that when autolytic events are causing the major changes in fish, i.e. during the first 7 days when stored on ice, K-value might be a sufficient indicator of freshness [41]. As storage time increases, however, the microbial degradation evolves, and K-value alone is not suitable any longer [41]. Several K-value indices have been reported, for instance, Erikson *et al.* (1997) proposed a lower K-value limit of 40-50% for Atlantic salmon of excellent quality, meanwhile the upper limit of good quality was set to 70-80 % [41]. Yet others report different levels; Saito *et al.* (1959) had the following categories: very fresh (<20%), moderately fresh (<50%) and not fresh (>70%) [40], whereas Ehria and Uchiyama (1974) and Lin and Morrissey (1994) suggested a rejection level close to 60% of products intended for processing [42, 43]. Furthermore, Japanese researchers defined a K-value limit for raw fish consumption of 20% [39]. It is clear that there exists a great range in K values and their quality indication. On the other hand, the mentioned values are based on different species; thus it may be reasonable to suspect that K-value quality levels are affected by this.

2.1.2 Spoilage of fish

Spoilage of fish has been defined by Mukundan et al. to be unacceptable changes occurring post-mortem in the fish muscle [44]. Spoiled food is often associated with off odour, slime and unpleasant taste. Fresh fish is highly perishable, and spoilage of fish is mainly caused by microbial activity [45]. Several other factors, illustrated in Figure 2.5, contributing to spoilage as well either by facilitating microbial growth, such as temperature and oxygen or by other deteriorating reactions such as fat oxidation.

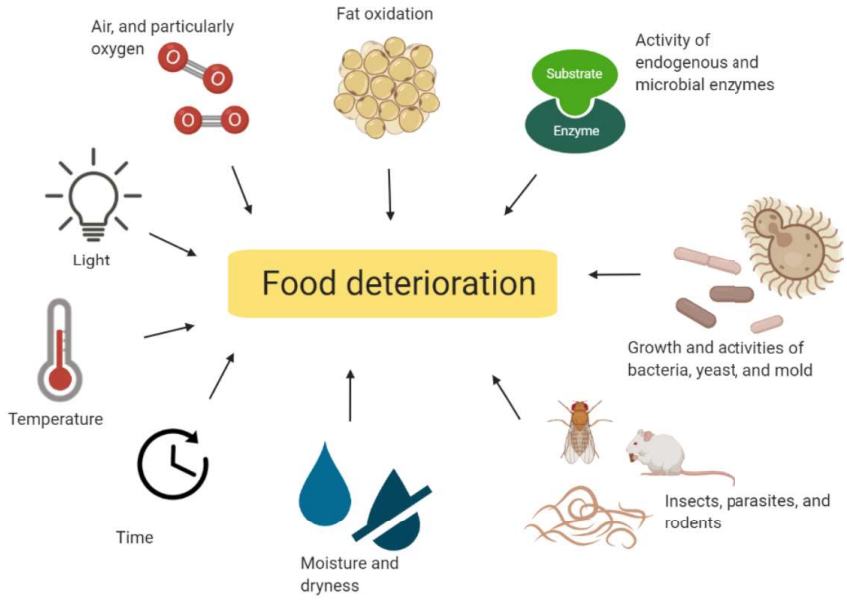


Figure 2.5: Overview of factors contributing to food deterioration. Created with biorender.com, and first published in the project report proceeding this thesis [46].

Gram et al. pointed out four parameters with great influence on the microbial spoilage: (1) the poikilotherm nature and the aquatic environment, (2) high post-mortem pH (usually > 6.0), (3) high levels of non-protein-nitrogen (NPN) and lastly (4) the presence of trimethylamine oxide (TMAO) [13]. The latter parameter is particularly prominent when oxygen is not present. Facultative anearobic bacteria can utilize TMAO as an electron acceptor in anaerobic respiration, thus converting it, by use of the enzyme TMA reductase, into trimethylamine (TMA), which is often associated with a strong fishy odour. The poikilotherm nature of fish allows bacteria with a broad temperature range to grow. High post-mortem pH allows pH-sensitive microorganisms, such as *Shewunella putrefaciens*, to grow. The NPN consist of low-molecular weight, water-soluble, nitrogen containing compounds, for instance, free amino acids and nucleotides, which are available as substrates for microbial growth. Two particularly important substrates regarding spoilage is the sulphur containing amino acids, cysteine and methionine. When utilized by microbes, known as H_2S-producing bacteria, the decomposition causes off-odours and -flavours as hydrogen sulphides and methyl mercaptane (methanethiol) is formed [13, 47, 48]. Consequently, H_2S-producing bacteria are classified as food spoilage organisms and spoilage levels have been reported from 3.5 - 5.3 Log CFU/g [49].

Although TMA is assumed to mainly be caused by spoilage bacteria, as TMAO reductase is of microbial origin, the correlation with the total bacterial count is often poor. The general opinion is now that the formation of TMA is caused by the presence of small numbers of specific spoilage bacteria (SSO). These bacteria do not necessarily amount to a large proportion of the total bacterial flora in newly processed seafood. However, they are characterized by the capability to produce large quantities of spoilage related compounds, for instance, TMA. *Shewunella putrefaciens* and *Photobacterium phosphoreum* are both common SSO capable of reducing TMAO to TMA [50],[51], [52]. The latter is also CO_2-resistant, making it one of the main spoilers of

modified atmosphere stored marine fish from temperate waters [13]. Lastly, the bacterial enzyme TMA oxidase is not solely responsible for the TMAO degradation. Endogenous enzymes can also cause decomposition of TMAO into TMA. [53]

The initial type of microorganisms present on the fish depends on fish species, environmental habitat, and contamination from the environment. Meanwhile, the dominating SSO species depends on factors such as packaging, conservation and temperature. When raw food is contaminated with a broad assortment of microorganisms, just a few is able to grow extensively, and a handful of these will be what is referred to as SSO [13].

In order to control microbial growth, eliminating oxygen or introduce CO_2 has been a common approach by use vacuum and MA-packaging. Sivertsvik et al. (2002) summarized four of the potential inhibiting effects of CO_2 on the microorganisms; (i) penetration of CO_2 in the membrane of bacteria causing intracellular pH changes, (ii) direct inhibition of enzymes or decrease their activity rate, (iii) Alteration of cell membrane function affecting the nutrient uptake and absorption, and (iv) direct changes in the physical and chemical properties of proteins [53].

When changing the surrounding conditions, the growth conditions are affected. Thus, the dominating microorganisms will be dependent on storage conditions. Although the use of CO_2 in MAP inhibits *Pseudomonas* spp. and *Shewanella* spp., it favours growth of gram-positive bacteria such as lactic acid bacteria, *Brochothrix thermosphacta* and *Photobacterium phosphoreum* [13]. As *Photobacterium phosphoreum* is resistant towards CO_2, it is not restrained by MAP to the same extent as *Shewanella putrefaciens*, which grows notably slower in the presence of CO_2 [54]. *Shewanella putrefaciens* is also pH-sensitive [13] and might be affected by the further decrease in post mortem pH caused by the solubilized CO_2 of MA packages. Moreover, as *Photobacterium phosphoreum* is capable of anaerobic respiration, it can utilize TMAO and form TMA. The H_2S-production of *Photobacterium phosphoreum*, however, seems to be strain-dependent [55].

Several studies have tried to address which SSO is responsible for limiting the shelf-life of salmon based on the storage conditions. Table 2.1 provides an overview of specific spoilage bacteria based on storage conditions.

Table 2.1: List of common spoilage bacteria in salmon stored under different conditions.

Air (aerobic)	MAP (CO_2 / N_2)	Vacuum (anaerobic)
Pseudomonas spp.[13] *Shewanella putrefaciens*[13]	*Photobacterium phosphoreum*[13, 56, 57, 58] Lactic acid bacteria;[56] e.g. *Carnobacterium maltaromaticum* [57] Psychrotolerant Enterobacteriaceae;[56] e.g. *Hafnia alvei*[57]	*Photobacterium phosphoreum* [49] Lactic acid bacteria; e.g. *Lactococcus piscium*[49] Psychrotolerant Enterobacteriaceae; e.g. *Serratia* sp.[49]

There exist no common fixed level concerning the microbial count needed to cause spoilage as it depends on species and packaging. Dalgaard (1995a, 1995b) reported that spoilage in MAP starts once the counts of *Photobacterium phosphoreum* reaches 10^7 cfu/g fish [59, 60]. However, Macé *et al.* (2013) evaluated the spoilage potential of eight bacterial species in MA packed salmon and

although the growth of *Photobacterium phosphoreum* was weak after 7 days of storage with a bacterial count of only 10^4 CFU/g, the samples appeared very spoiling [57]. Moreover, Macé et al. (2012) suggested that there is a shift of the dominant microbiota during storage. *Pseudomonas spp.* dominated in the beginning, while *P. phosphoreum* and *L. piscium* were the main species at 7 and 10 days after storage [49]. Nevertheless, in some cases, the correlation between counts and spoilage may be poor and the end of shelf-life has in some MAP products been determined by sensory rejection, while microbial counts are still below spoilage levels. The end of shelf-life has, in these cases, been driven by non-microbial degradation causing sensorial changes at a point of rejection. Thus, a majority of studies include sensory assessment in order to provide a proper shelf-life proposal [61].

As APC may be a poor indicator of spoilage, other parameters have been proposed. Dalgaard *et al.* (1993) reported spoilage levels of SSO, where the aerobically, iced store fish required levels of 10^8-10^9 cfu/g of SSO, while spoilage of chilled, CO_2 packed cod had a limit of 10^7 cfu/g [52]. However, the amount of spoilage related compounds, caused by SSO, depends on the bacterial species. For instance, *Photobactetium phosphoreum* produces on average 30 times the amount of TMA compared to *Shewanella putrefaciens* [60]. Consequently, the amount of SSO needed to cause spoilage is species dependent. A different approach to assist shelf-life determination is by monitoring certain nucleotides from the ATP degradation. As previously stated, microbial enzymes are assumed to be the main contributor when degrading In into Hx. [36] In order to determine spoilage, a statement referred to as H-value [62] can be used and as been defined by Luong *et al.* (1992) as

$$H - value(\%) = \frac{[Hx]}{[IMP] + [In] + [Hx]} \cdot 100 \tag{2.2}$$

Accumulation of Hx results in the loss of desirable flavors and the product may appear spoiled [12]. The H-value differs from K-value by explicitly expressing quality decline in terms of spoilage, not freshness, and Luong *et al.* (1992) reported that no correlation between H-value and fish freshness exist. Thus, H-value is an indicator of spoilage as it indicates bitter off-taste caused by the microbial activity [62].

As there have been little quantitative analyses on H-values and the corresponding level of spoilage, it may be challenging to address the shelf-life based on H-value alone. The lack of H-value indices has received limited attention in literature, however, Luong *et al.* (1992) reported K- and H-values of freshly caught lake salmon, see Figure 2.6 [62]. However, the raw-material was frozen prior to testing. The nucleotides remain intact when frozen, whereas the microbiota is affected. The microbiota of MAP is especially affected as *Photobacterium phosphoreum* dominates the spoilage microbiota and is eliminated by freezing [58]. Hence, freezing may affect which type of SSO will dominate and particularly affect the H-value as it is mainly determined by the activity of the present microbial enzymes.

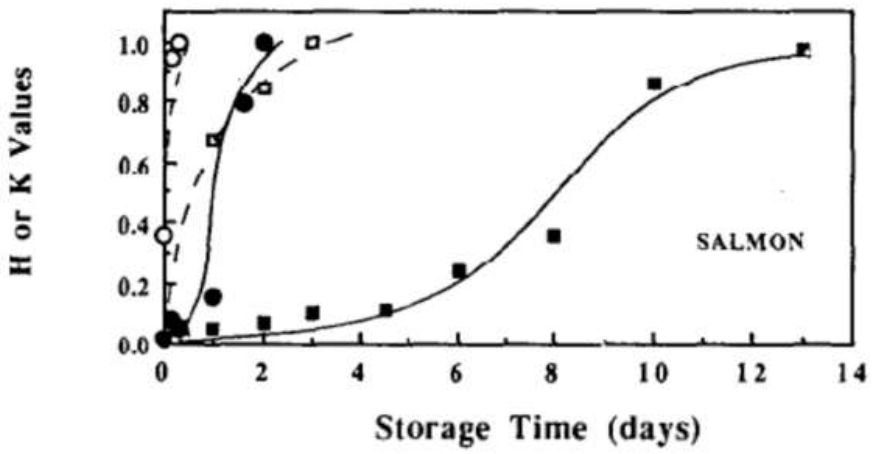


Figure 2.6: Change of H-value (■, 4°C; ●, 20°C) and K-value (□, 4°C; ○, 20°C) throughout storage, determined by capillary electrophoresis. The graph is collected from [62].

2.1.3 Pre- and post-rigor filleting

ATP depletion, and consequently the onset of rigor mortis, is highly correlated to the residual glycogen level, as described in previous sections. Thus, exhaustion and vigorous movement during capture result in earlier onset of rigor mortis. As a result, the traditional fishing industry has primarily used post-rigor filleting. In aquaculture, however, Skjervold *et al.* (2001) demonstrated that certain harvesting techniques could postpone rigor mortis to the extent that pre-rigor filleting can take place [63].

The time perspective associated with pre-rigor filleting is an important advantage compared to post-rigor filleting. The resolution of rigor usually takes 3 to 7 days after slaughter [64]. However, when filleting pre-rigor, which is only hours after slaughter, the fish can be packed and transported immediately after. The Ready-to-Eat products from FRØYA guaranty a maximum of two hours from sea to sealed, meaning from the salmon is alive until vacuum packed and ready for transport. [65] According to Hansen *et al.* (2009), pre-rigor filleting improves logistics at the processing plant and eliminates costs associated with the transport of head and bones. Furthermore, as the fish reach the market 3-7 days earlier, it allows super-fresh products to enter the market [66].

Managing Director at Vitenparken Science Center states that when applying pre-rigor filleting, the salmon products will become available for the consumers in Tokyo before the post-rigor filleted fish has been filleted in Norway. The time perspective is a great advantage and makes pre-rigor filleting a popular approach for long-distance shipping. As the technique has become practically feasible, and there is a possibility of economic benefits, the interest in the method has increased notably in the last decade [67].

An unfavorable effect of pre-rigor filleted fish is the shortening of the fillet. Fillets have shown

to shorten by up to 8 % due to the absence of backbone during rigor contractions. However, the absent backbone reduces the risk of gaping as the fillet is not restrained by it [63]. Rosnes *et al.* (2003) reported lower gaping and higher fresh odor scores, and Skjervold *et al.* (2001) reported a significant reduction in incidence and severity of gaping with earlier filleting as well as a positive effect on visual color. The water loss, however, was greater for pre-rigor fillets [68].

2.2 Preservation and Hurdle technology

In order to maintain the edibility of fish as long as possible after slaughter, several measures can be taken. Preservation is crucial to ensure safety, as well as the sensory and nutritional quality of food. Furthermore, by combining preservation factors, i.e. hurdles, the microbial stability and safety of food are maintained. The microorganisms are inhibited as they are exposed to a hostile environment, which either constrains the growth or causes death. "The most important hurdles used in food preservation are temperature (high or low), water activity (a_w), acidity (pH), redox potential (Eh), preservatives (e.g., nitrite, sorbate, sulfite), and competitive microorganisms (e.g., lactic acid bacteria)" [69]. In order to modify the given parameters, different hurdles are needed. Table 2.2 illustrate the context.

Table 2.2: Preserving parameters and their respective hurdles [70, 71].

Parameter	Symbol	Hurdles
High temperature	F	Heating
Low temperature	T	Chilling, freezing
Reduced wateractivity	a_w	Drying, curing, conserving
Increased acidity	pH	Acid addition or formation
Reduced redox potential	E_h	Remove oxygen or addition of ascorbate
Biopreservatives		Competitive flora (e.g. microbial fermentation)
Other preservatives		Sorbates, sulfites, nitrites

2.2.1 Process and storage temperature

Both autolytic changes and microbial spoilage are highly affected by temperature. The growth rate of bacteria and the enzyme activity with respect to temperature is illustrated in Figure 2.7 (a). Temperature between 0 to 25°C has a greater influence on microbial activity than enzymatic activity. When temperatures go below 10°C, most of the microbial activity is notably reduced, and even psychrotrophic organisms grow at a slow rate. Thus, spoilage linked to unpleasant odour and taste caused by microbial enzymes could be postponed by restraining the microbial growth with low temperature during storage.

As stated before, the shelf-life of fish is highly dependent on the microbial growth of SSO and can be notably extended if stored at low temperatures. The growth rate of the spoilage organism *Shewanella Putrefaciens* with respect to temperature under aerobic and anaerobic conditions are

illustrated in Figure 2.7 (b). By limiting the access to O_2, the growth rate of *S. Putrefaciens* is reduced. However, as described in the previous section, under such conditions, the typical spoilage component, TMA, is more likely to be formed. Furthermore, it is obvious that with increasing temperatures towards 30°C, the growth rate is increasing. Thus, it is important not to rely solely on hurdle technology, but combination with low storage temperature to postpone microbial spoilage [72].

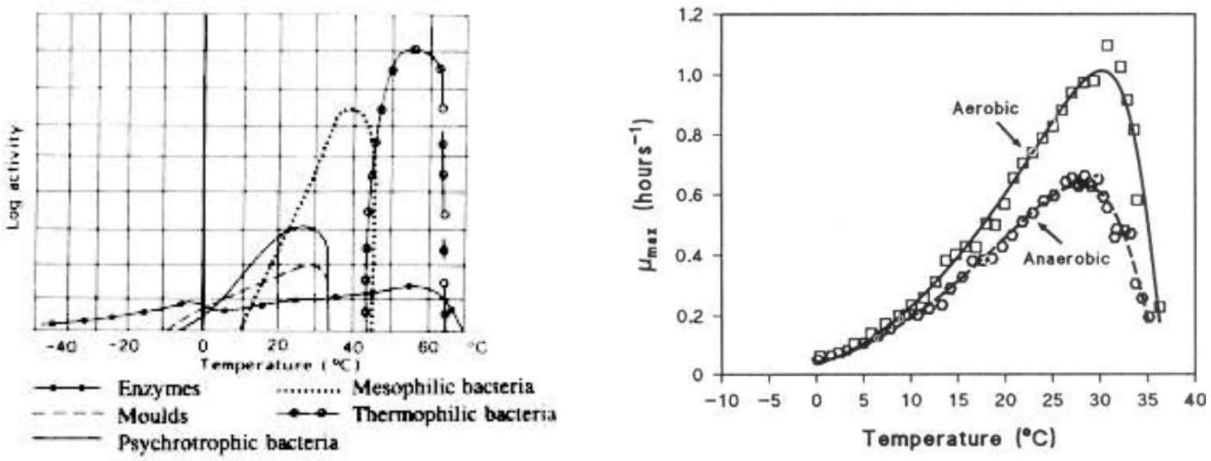


Figure 2.7: (a) The enzyme activity and growth rate of microorganisms with respect to temperature (°C) [73]. (b) Illustration of the specific growth rate, μ_{max}, of *Shewanella putrefaciens* as a function of temperature (°C). *Shewanella putrefaciens* was grown on a complex medium containing TMAO [74].

Traditional preservation techniques, such as salting, drying, smoking, and freezing, change the initial product tremendously. When food is preserved as mentioned, it cannot be regarded as fresh any longer. To meet the consumers' demand for fish that retain the sensory and nutritional qualities of fresh fish for an extended period of time, other methods had to be developed. Vacuum and modified atmosphere packages (MAP) are two techniques that have been extensively used across the world. These techniques extend the shelf-life while preserving the freshness [75]. Both vacuum and MAP eliminate the oxygen access, thus affecting the aerobic microorganisms eminently. Additionally, CO_2 is introduced in the MA packages, which further constrains the microbial growth [76].

2.2.2 Principles of vacuum

Vacuum can be classified as a type of modified atmosphere packaging as major parts of gasses surrounding the product is removed, consequently changing the atmosphere. As gasses are not re-introduced, it is more commonly known as vacuum. In this thesis, vacuum and MAP will be referred to as two distinct packaging methods. By not re-introducing gasses, vacuum has a great advantage as the packed product has approximately the same volume as the food itself. A degree of filling close to 100 % is both economical and environmentally friendly as less fuel is used in transport per kilo of food. Nevertheless, vacuum has its disadvantages, making it

unsuitable in some cases. First, there is the physical impact on the food caused by the imploding plastic. Second, the resistance towards microbial growth is limited. Oxygen is absent, however, in contrast to MAP, no CO_2 is present to inhibit bacterial growth further. As carbon dioxide is absent, vacuum facilitates the growth conditions of anaerobic organisms, thus increasing the risk for highly unwanted microorganisms. Furthermore, the aerobic bacteria are not entirely inhibited as a certain amount of oxygen will remain in the vacuum package [75].

2.2.3 Principles of MAP

The key factor of modified atmosphere packaging is the gas atmosphere, especially the concentration of CO_2. It is a well-established fact that the headspace CO_2 in modified atmosphere packaging inhibits the microbial growth in fish products [52, 53, 77–79]. The headspace gas composition consists of large amounts (25%-100%) of CO_2. By packing food in modified atmospheres, the shelf-life has shown to increase by 50-100%, due to the inhibiting effect of CO_2 [53]. However, it is widely recognized that MAP cannot improve quality or give back lost shelf-life. Accordingly, MA technology should not be utilized as a substitute for poor sanitation or high temperatures.

The inhibiting effect of headspace CO_2 on microorganisms occurs when the gas dissolves in the food matrix. CO_2 is highly soluble in the water and fat content of food and dissolves according to the following equation;

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \tag{2.3}$$

Equation 2.3 has been modified and is typical for seafood. Seafood has often pH values below 8, thus the further formation of carbonate ion (CO_3^{2-}) is neglected [79].

An initial 60/40 mixture of CO_2 and N_2 is common for fatty fish, such as salmon. After sealing, there is no control of the gas mix, and the gas concentrations surrounding the product will change as CO_2 dissolves into the product, consequently increasing the partial pressure of nitrogen, which is less soluble. Furthermore, when CO_2 dissolves into the tissue, a volume contraction of the semi-rigid MA-package may occur. Nitrogen is an inert gas that is present in order to replace oxygen, and at the same time prevent packaging collapse, often known as snug down. In order to avoid snug down, sufficient amounts of gasses which do not get absorbed bu the product are necessary [80]. Nitrogen is often used due to its low solubility in fat and water, thus making it suitable as filler gas. Figure 2.8 illustrates the solubilities of nitrogen and carbon dioxide in water with respect to temperature.

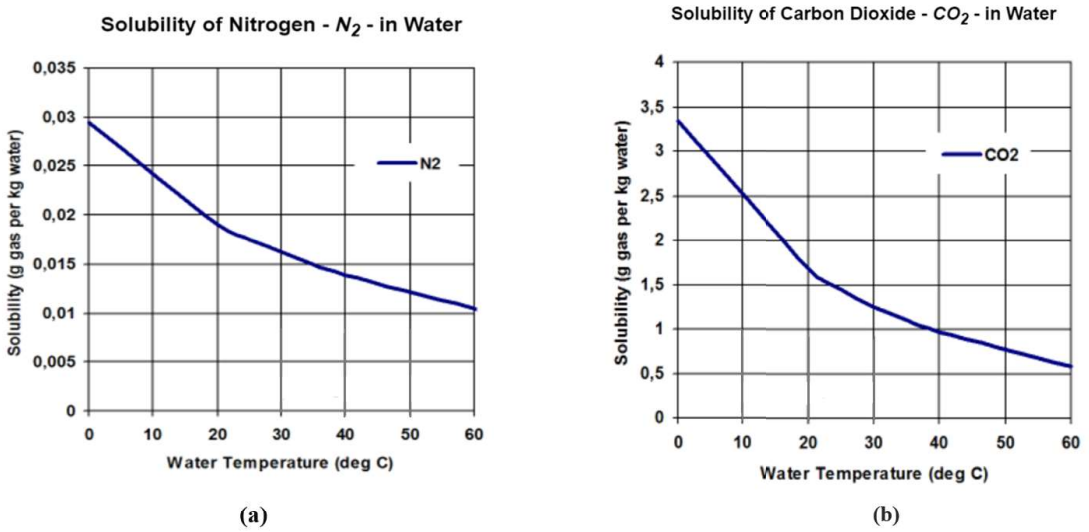


Figure 2.8: (a) Amount of dissolved N_2 in water as a function of water temperature, C°. (b) Amount of dissolved CO_2 in water as a function of water temperature, C°. The solubilities of N_2 and CO_2 in water are given as g gas per kg water. The graphs were collected from [81].

As seen from the graphs in Figure 2.8, the solubility of CO_2 decreases markedly with increasing temperatures. Sivertsvik *et al.*(2002) and Rotabakk *et al.* (2012) stated that the bacteriostatic effect is proportional to the amount of dissolved CO_2 in the product [53, 80]. Thus, the effect of MA-packages is limited to the amount of available CO_2, which can dissolve into the product. Consequently, low temperatures are crucial for the shelf-life of MAP products [53]. Furthermore, the amount of dissolved CO_2 can be increased by increasing the partial pressure of CO_2 (P_{CO_2}) in headspace. An increase in P_{CO_2} can be accomplished by either (1) increasing the headspace concentration of CO_2, or (2) increase the gas volume to product volume ratio (g/p ratio) [82].

The exclusion of O_2 is recommended for fatty fish to avoid rancidity as the presence of oxygen accelerates the process. In the case of lean fish, a mix of 30 % O_2, 40 % CO_2 and 30 % N_2 is common. Oxygen is re-introduced to avoid the anaerobic reduction of TMAO into TMA [83], as certain microorganisms utilize TMAO as the terminal electron acceptor when oxygen is not available [84]. In order to avoid the formation of TMA, which smells of spoiled fish, some oxygen is favorable in cases where the risk of rancidity is small, such as for lean fish.

Although MAP comes with many beneficial effects, some concerns have been expressed as there is an adverse effect when changing the atmosphere as the microbiota changes due to changes in the growth conditions. Even though the alterations restrain spoilage bacteria, some pathogenic bacteria may arise, which under regular conditions would not grow extensively. For instance, the anaerobic *Clostridium botulinum* type E can grow in typical MAP conditions and produces toxin in low-oxygen atmospheres at refrigerated temperatures [53], [85]. Furthermore, the genera *Clostridium* is also an endospore-forming organism, consequently tolerating high pressure or temperatures. Certain *Clostridium* species are important for human health and physiology [86, 87], however some is pathogenic and highly unwanted in food such as *Clostridium botulinum,*

C. difficile, C. perfringens, and *C.tetani* [88–91]. In general, all harmful endospore-forming organisms are highly unwanted in food as their spores are not inactivated by cooking and may cause severe health problems [92].

2.2.4 Improving existing technology

Numerous types of hurdles are available, and new techniques with better properties are continuously developed. Traditional hurdle techniques can be combined with more recent technology, such as SGS, CO_2 emitters and oxygen absorbers, to optimize the storage conditions by eliminating oxygen and introducing CO_2. For instance, Sivertsvik (2000, 2003) reported promising results on extending shelf-life of fresh Atlantic salmon (*Salmo salar*) fillets by the use of SGS-processing [76, 93].

Soluble Gas Stabilisation (SGS)

As previously stated, CO_2 suppresses the microbial growth. In order to maintain the bacteriostatic effect, while at the same time prevent snug down, a low DF is needed to withhold a high CO_2 concentration in headspace. As CO_2 dissolves into the food matrix, the amount of CO_2 in headspace will decrease. Eventually, the bacteriostatic effect ceases as low amounts of CO_2 are available in headspace for solubilization. The SGS-technique involves dissolving CO_2 into the product prior to packaging. As found by Rode *et a.l* (2015) this technique results in high amounts of CO_2 in headspace throughout storage compared to MAP [94]. As the initial headspace concentration of CO_2 is maintained as the food is saturated prior to packaging, the risk of packaging collapse associated with high headspace CO_2 concentrations is eliminated. This was confirmed by Al-Nehlawi *et al.* (2012), who reported packaging collapse to be significantly reduced in SGS samples [95].

SGS is a novel approach with a potential of (1) counteracting the decrease of headspace CO_2 and/or (2) reduce the package size. By counteracting the decrease of CO_2, it is believed to enhance shelf-life by maintaining the bacteriostatic effect while keeping the original DF. Secondly, it is proposed that a higher DF can be applied before snug down occurs and without compromising the bacteriostatic effect. Conventional MAP is space demanding due to the low DF. By applying SGS prior to packaging, the product is saturated to a certain extent, accordingly reducing the flux of diffusion after sealing and preventing snug down. Consequently, SGS provides a better packaging efficiency as more product can be transported per volume, as well as reduce the amount of required packaging and lastly, the potential of increasing shelf-life.

CO_2 emitter

Another approach to solve the lack of CO_2 has been by use of CO_2 emitters. Due to their production of CO_2, the emitters can compensate for an increased DF. The emitters have shown promising results and recent years Hansen *et al.* (2015) found the shelf-life of vacuum, as well as MAP with low headspace volume, to be prolong by 2-4 days [96]. Even though the developed CO_2 gas from the emitter was absorbed into the fish, hence the product appeared like an ordinary vacuum packaging, it has been previously reported several weaknesses by Hansen *et al.*(2009) when an

attempt was made to combine CO_2 emitters and MA-packages, for instance too high or too low amounts of developed CO_2 [97].

Pros and cons of novel packaging techniques

A disadvantage associated with the SGS-technique is the time consuming process of dissolving CO_2 into the product. It can take up to several days to reach equilibrium. Rotabakk (2008) reported that the time to reach 50% dissolved CO_2 in chicken breast fillets varied from 7.7 - 9.3 hours, and 97% was reached after 39 - 47 hours [9]. Consequently, when utilizing SGS to dissolve CO_2 into the product prior to packaging, the time from sea to sealed increases notably. When using a CO_2 emitter, the dissolution of CO_2 occurs after packaging. Hence it is less time consuming as the products can be transported while being saturated with CO_2.

However, there exist several challenges associated with the highly elevated CO_2 concentrations, concerning both SGS and CO_2 emitters. First, the acidifying effect of solubilized CO_2 decreases the pH, thus enhancing exudation as the water holding capacity of the fish proteins is reduced [98]. Secondly, salmon, which contain carotenoid pigments, may become paler during prolonged storage in CO_2 enriched atmospheres [99]. Lastly, with such high amounts of solubilized CO_2 there is a risk of getting a tingling sensation on the tongue when eaten raw. However, the latter is most likely not a problem if cooked as the heating process will release the dissolved carbon dioxide from the tissue. On the contrary, this effect has not been thoroughly investigated. Hence, to determine if, for instance, SGS is applicable to Ready-to-Eat products, further testing must be conducted.

Though there are some drawbacks associated with SGS, there are many beneficial aspects compared to other CO_2 enhancement methods. As previously mentioned, vacuum is desirable in terms of DF, yet it has a lower bacteriostatic effect than other barrier technologies. This can be improved by the use of SGS prior to vacuuming, whereas CO_2 emitters may have a greater risk of developing excessive gas. If this occurs, it will be difficult to tell by the consumers and/or grocery store if the package has ruptured, allowing air into the packaging, or if it is CO_2 gas surrounding the product. This can, however, be a problem in the case of SGS-vacuum as well if the dissolved CO_2 in the tissue diffuse out. Yet, of the >100 samples in this experiment, only one of the vacuum samples had CO_2 gas surrounding the product, which had diffused out from the tissue. Furthermore, to my knowledge, no instances concerning this issue have been previously reported. Thus, it seems not to be a pervasive problem.

2.3 Quality parameters of salmon

Quality is an arbitrary term and can often be subjective. Consequently, quality can be a rather vague description causing confusion. In order to make the term less complex, several parameters have been defined. According to Sigurgisladottir *et al.* (1997), the following quality parameters were particularly important for salmon;

Table 2.3: Quality parameters based on Reviews in Fisheries Science vol.5, Salmon quality: Methods to determine the quality parameters [100].

Parameters*	Description
Fat content	Composition, distribution
Color	Intensity, distribution
Texture	Firmness (hardness/elasticity), gaping
Others	White stripes (connective tissue), bleeding blood stains, marble, melanin

*"Fat, pigments, and collagen are distributed differently over the salmon fillets. Consequently, the numerical values of the quality parameters are different depending on where the sample is collected from the fillet." [100]

2.3.1 Color

The following section has been modified and re-written based on the project report published prior to this thesis [46].

Carotenoids is one of the main pigments in salmon and rainbow trout as it contributes to the desirable and highly attractive red, orange and yellow color. These pigments, however, are easily oxidized in seafood by a lipogenase-like enzyme [101]. The red color of salmon is caused by the carotenoid astaxanthin and is linked to several positive effects on the defense against stress and diseases in mammals [102]. Salmon do not produce astaxanthin but are colored through diet by eating crustaceans that eat astaxanthin producing algae [102]. Synthetic astaxanthin is supplemented in the diet of aquacultured salmon as a compensate for the astaxanthin wild salmon consume from the algae [103].

One of the first quality parameters the consumers' faces when buying salmon fillets is the color. A study on the effect of color on consumer willingness to pay (WTP) for farmed salmon revealed a correlation between color and WTP. According to Steine *et al.* (2005), consumer WTP increases with the redness of the salmon [104]. Consequently, the price per kg is positively correlated to the amount of redness [105]. Furthermore, the Norwegian Seafood Research Fund (FHF) even stated as one of their objectives to perform research on which conditions that ensure good coloration, thus emphasizing the importance of color [106].

As with all quality parameters, the color is also greatly affected by handling, storage and processing. Extrinsic factors such as temperature and atmosphere, as well as intrinsic factors such as post-mortem changes, influence the appearance. Erikson and Misimi (2008) concluded that color changes are affected by the onset and development of rigor mortis, post-mortem glycolysis, and pH drop. These parameters affect the muscle- and cell structure, resulting in changes in the light-scattering properties. Consequently, changing the appearance of the salmon [107]. Furthermore, there have been indications that fish stored under high concentrations of CO_2 over a longer period of time may lighten [108]. Additionally, the red color is especially sensitive to

temperature, Hiltunen *et al.* (2002) found changes in color when the temperature varied with just a few centigrades [109].

The color can be measured using both imaging and physical measurement tools. Physical tools can be subjective, thus imaging tools are used to obtain a completely objective color determination of an item. Imaging instruments define color by a*-value (red/green), b*-value (yellow/blue) and L*-value (lightness). [110] These three elements can be visualized in a three dimensional system, as illustrated in Figure 2.9.

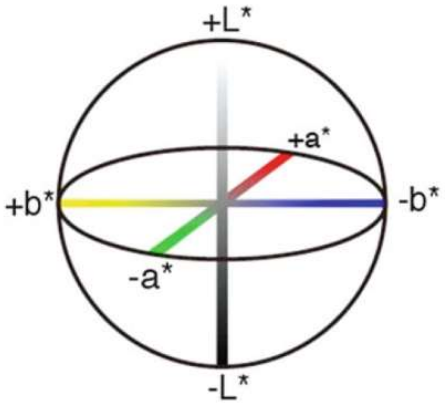


Figure 2.9: A three-dimensional illustration [111] of the CIElab color system of L*, a* and b* coordinates [112]. Copyright(2014) The Japan Society of Applied Physics.

Chapter 3

Materials and methods

This section outlines the materials and methods applied in the study. The section was originally published in the project report preceding this thesis. Additionally, minor alterations have been done [46].

The salmon used in the experiment was slaughtered and collected at Lerøy Midt AS located on Jøsnøya. The fish was kept on ice during transport to prolong the state of pre-rigor. The pH was registered approximately 5 hours after slaughter to 6.7 ± 0.3 in fish intended for pre-rigor samples. The fish was filleted into samples before exposed to CO_2 for approximately 21 hours at 4°C with no ice and repacked into MAP and vacuum as described in section 3.1. Pre-rigor salmon intended for post-rigor filleted samples were stored head on gutted (HOG) at 4°C on ice for 4 days by which rigor mortis had ceased off. The pH in post-rigor salmon was registered before filleting to 6.53 ± 0.13 and a core temperature of 1.9 ± 0.1°C. The post-rigor filleted salmon was processed, packed and stored in the same manner as the pre-rigor filleted salmon. The SGS-system bags with CO_2 had a gas composition after saturating fillets for 21 hours of 95.6 ± 0.2 % (pre-rigor filleted samples) and 96 ± 1 %(post-rigor filleted samples).

Samples were analyzed regularly during a period of four weeks until seven sampling points were accomplished. The aerobic microbial counts, H_2S producing bacteria counts, headspace gas composition and pH were investigated at each sampling, whereas ATP degradation compounds were monitored 3, 7 and 11 days after filleting. Additionally, color assessments were performed on raw material and 11 and 30 days after filleting.

3.1 Experimental design

Half of the collected pre-rigor salmon was filleted, meanwhile the rest was stored for 4 days on ice at 4 °C, awaiting the resolution of rigor mortis. Outer parts were cut off during filleting to obtain uniform lipid levels in the samples.[1] The fillet pieces of 80 ± 1g were separated into two groups; SGS and control. The control group was not processed with CO_2 prior to packing. All the samples were packed into plastic bags. The SGS-bags and control bags were inflated with

[1] See Figure A.1 in Appendix as an illustration of the lipid distribution of salmon fillets.

CO_2 and air, respectively, and sealed subsequently using Single Chamber Machine SuperMax-C, WEBOMATIC. After approximately 21 hours in the closed systems at 4°C, the samples were repacked. Both groups were divided once more, resulting in four different groups. Half of the SGS treated samples were repacked into 230 ml CPET trays using WEBOMATIC Tray Sealing Machine TL250, with gas composition 40/60 (%) of N_2 and CO_2, respectively. The other half of the SGS-treated samples were vacuumed using SuperMax-C. The control groups were repacked into MAP and vacuum in the same manner and the following four groups were obtained; SGS-MAP, SGS-vacuum, MAP, and vacuum. A flow sheet of the processing set-up is given in Figure 3.1,

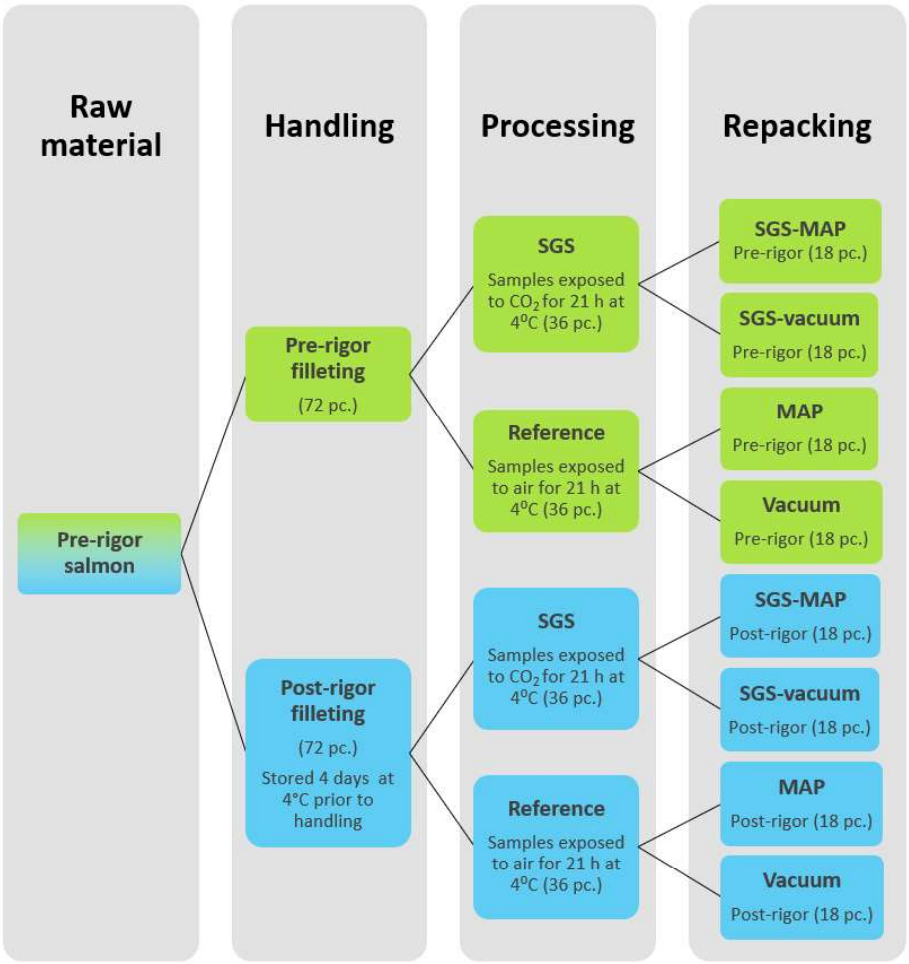


Figure 3.1: A flow-sheet illustrating the treatment process of pre-rigor and post-rigor salmon. Pre-rigor filleted salmon was divided into two groups; one was treated with carbon dioxide, the other with air. After the saturation of the samples with the respective gasses, the groups were divided in half again and repacked to vacuum and modified atmosphere packages (MAP), making a total of four groups with different treatment processes. The gas composition in MA-packages was set to 40% N_2 and 60% CO_2. The post-rigor filleted salmon was treated in the same matter four days after.

3.2 Storage and sampling

The samples were kept in a fridge holding a temperature of 3°C. Three parallels per group were withdrawn at each sampling point. An overview of sampling days of pre- and post-rigor filleted samples is given in Figure 3.2. The samples were randomized to ensure that different fish and parts were analyzed simultaneously.

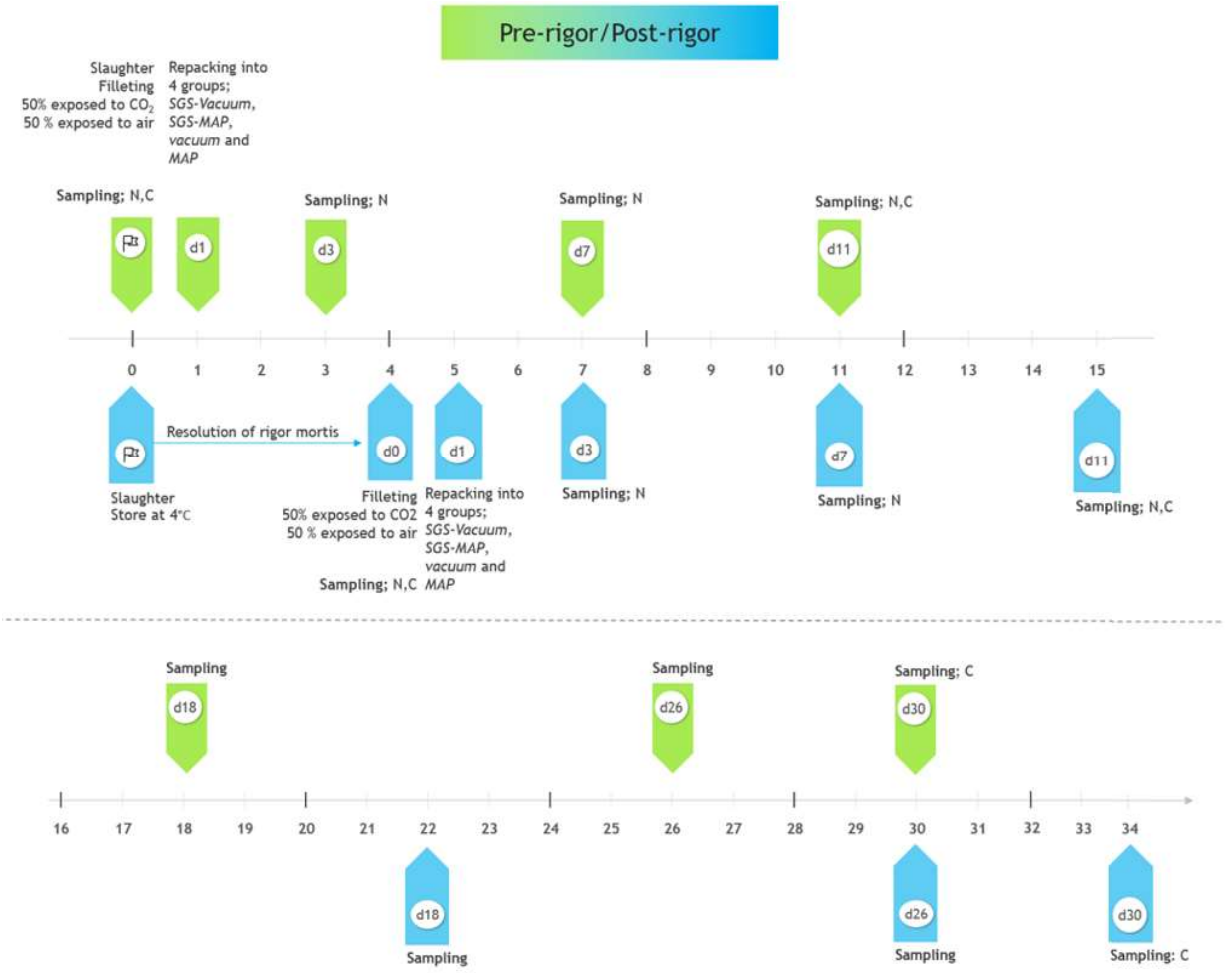


Figure 3.2: An overview of sampling days of pre-rigor filleted (green) and post-rigor filleted samples (blue). Post-rigor filleted samples had a four days delay due to the resolution of rigor mortis. At each sampling, three parallels were withdrawn from each of the pre- and post rigor filleted groups; MAP, SGS-MAP, vacuum, SGS-vacuum. The aerobic plate count was determined at each sampling, meanwhile ATP nucleotide degradation compounds (N) were monitored 0, 3, 7 and 11 days after filleting, with 0 indicating raw-material. Color measurements (C) were performed on raw-material and samples from 11 and 30 days after filleting.

The data presented in this thesis are based on time elapsed after filleting. As post-rigor filleted samples were stored for 4 days during the resolution of rigor, age since slaughter differ between pre- and post-rigor filleted groups. For pre-rigor filleted samples, days after slaughter and days after filleting are the same, meanwhile time after filleting of post-rigor filleted groups have a 4

days delay. In this thesis, the comparison of quality and composition is based on time of filleting, unless stated otherwise. Furthermore, plots of ATP degradation compounds based on time after slaughter is given in Appendix 6.5.

A schematic illustration of sample preparation and sample analysis is given in Figure 3.3. Different measurements were applied on separate parts of the sample, demonstrated in Figure 3.3 (ii).

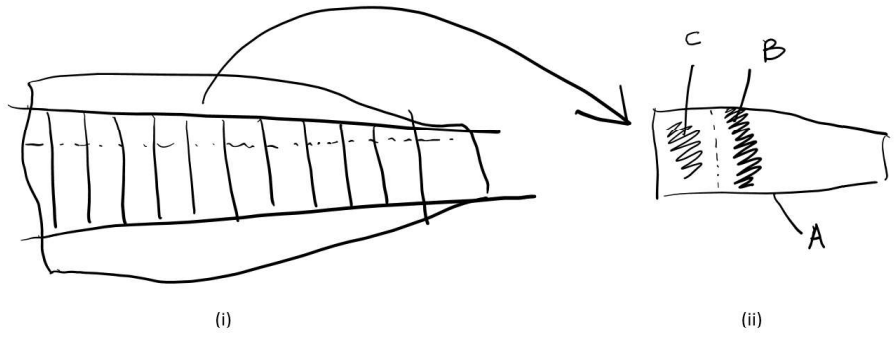


Figure 3.3: (i) Illustration of sample preparation from salmon fillet. (ii) Illustration of sample and sections used for analysing. At each sampling point, the samples were pH tested in region A, followed by microbiological characterization using section B. Color assessments were performed in region C.

Headspace gas analysis

The headspace gas composition of SGS-MAP and MAP was analyzed each sampling day by using Checkmate 9900, PBI Dansensor. Additionally, control analyses of dummies were performed during packing. The gas analyzer measured the CO_2 content and the residual O_2 content. Enclosed septums were applied to the packaging film to ensure that measurements were not affected by leaks occurring from penetration of the needle through the packaging film.

Muscle pH

The pH was registered using Testo 206 pH2. The pH meter was adjusted before use at each sampling point by a two-point calibration at pH 4 and 7.

3.3 Microbial analysis

Media to identify APC and H_2S-producing bacteria were used to evaluate the microbial characteristics and detect spoilage bacteria. Samples were diluted with peptone water to 10^{-1} and 10^{-2} at the first sampling. The dilutions were altered concurrently with the increasing amount of microbes. Counts of zero were set as log(1) prior to plotting to avoid undefined numbers.

Homogenates of fish samples were used to quantify the APC and H_2S-producing bacteria. Samples of 10 g fish muscle were homogenized 1:10 with peptone water (8,5 g/L NaCl and 1,0 g peptone) to a liquid suspension using a stomacher for 60 s. Further dilutions were prepared by using sterile peptone water. An illustration of the plating is given in Figure 3.4.

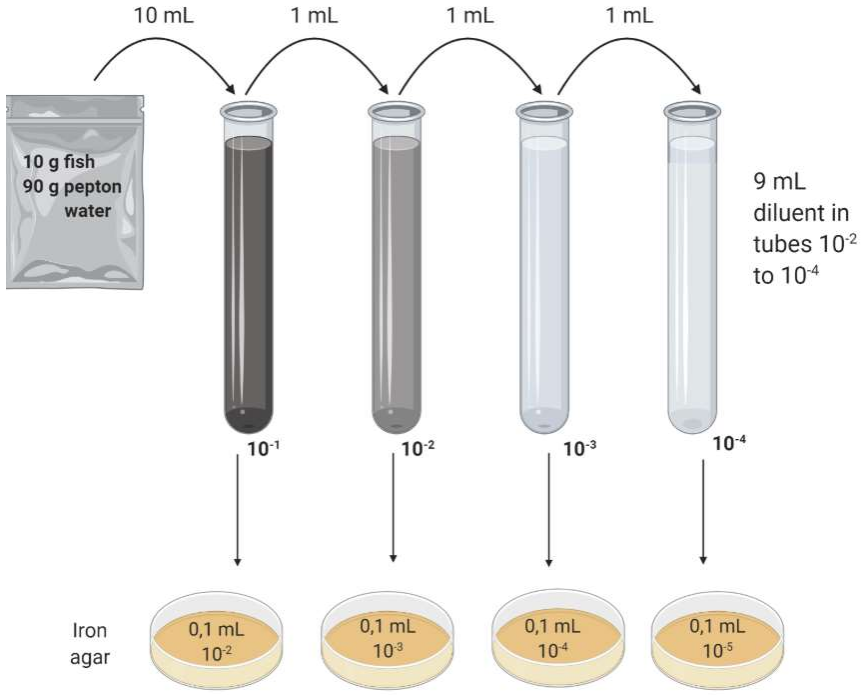


Figure 3.4: Schematic illustration of the experimental set-up of the dilution series and the plating. The iron agar medium obtained a dilution factor one unit higher than the dilution-tube. Thus, the lowest, obtainable dilutions for iron agar is 10^{-2}. Created with Biorender.com

Determination of APC and H_2S-producing bacteria was conducted according to the NMKL-method no. 184. [113] Lyngby Iron agar (CM0964), Oxoid ltd. with the addition of L-cystein, Sigma-Aldrich Co. The method is a quantitative determination of non-heat labile bacteria due to pour plating of 45°C iron agar. Aliquots of 1 mL from the dilutions were mixed with melted and temperated (45°C) iron agar and a top layer was added subsequently. The plates were incubated at 23 $\pm$ 2° C for 3 days. Black colonies were H_2S-producing bacteria, and the total aerobic plate was composed of both the black and white colonies.

3.4 Color analysis

The digital color imaging system, DigiEye, VeriVide Ltd., Leicester, UK, was used to measure the surface color of raw material, *n=5*, at day 0 and samples, *n=3* for each group, at 11 and 30 days after filleting. The test area was manually selected, and measurements were performed above the lateral line, as seen in Figure 3.3. The color data obtained from DigiEye system was processed using the software Digipix to transform the RGB values into the CIE values L* (lightness), a* (redness), and b* (yellowness).

3.5 ATP degradation products

At each sampling point, the samples were frozen at -80 °C after the microbial analysis to be analyzed afterward by HPLC.

Frozen samples of pre-rigor and post-rigor from day 3, 7, 11 and raw material were finely chopped to 1 g by using a grater and mixed with 7,5 mL of trichloroacetic acid, TCA, (7% w/v). Liquid suspensions were obtained by mixing the samples at 12 000 rpm for 1 min using IKA - T25 digital ULTRA TURRAX®. The mixing tubes were kept on ice during mixing to avoid heat generation. After mixing, 3250 μL of Potassium hydroxide, KOH, (1 M), was added to achieve a pH of 6,25. All samples were duplicated to minimize errors. The suspensions were kept on ice during preparation, followed by centrifuging using Rotina 420R, Hettich Zentrifugen at 1800 rpm for 15 min at 4°C. Cloudy samples were centrifuged again to avoid filter clogging. Approximately1 mL of the supernatant was filtered into HPLC tubes, using a 25 mm Syringe Filter w/0.2 μm polyethersulfone membrane. The prepared samples were frozen at -80 °C until further analysis with HPLC.

Samples were defrosted and run through the HPLC system. The HPLC system was composed of an Agilent 1290 attached to an infinity Diode Array detector (Agilent Technologies). An 120 column Poroshell (EC-C18, 3.0 x 100mm, pore size: 2.7 μm, Agilent Technologies) with a Poroshell 120 precolumn (3.0 x 5mm, Agilent Technologies). The mobile phase consisted of dihydrogen caliumphosphate (KH_2PO_4, 0.215 M) and tetrabutylammonium hydrogen sulphate buffer (CH_3CN, 0.0023 M, with 3.5% Acetonnitrile as diluent). The pH of the mobile phase was adjusted to 6,25 with 1.0 M KOH). Different flow rates were used during the run of 10 min. The flow rate was set to 0.2 mL/min the first two minutes (0-2 min), followed by a flow of 0.8 mL/min from 2-9 min, and finally 0.2 mL/min from 9-10 min. The column had a temperature of 20°C. The ATP degradation products were detected at 210 nm (ATP, ADP) and 260 nm (AMP, IMP, In, and Hx),

To identify the metabolites in the samples, standard solutions of each metabolite were first used to determine the retention time, R_t, associated with each ATP degradation product. The samples were run through the HPLC column and identified by comparing the peaks to the retention time. The retention time, R_t, of ATP, ADP, AMP, IMP, In, and Hx, was determined by running a 200 $\mu mol/L$ solution of each metabolite, with 3.5% Acetonitrile as the diluent, through the column.

Furthermore, standard solutions of different concentrations were used to calculate the amount of each metabolite by making a standard curve. The standard solutions of the individual metabolites had concentrations of 1000 $\mu mol/L$, 500 $\mu mol/L$, 250 $\mu mol/L$ and 125 $\mu mol/L$ with 3.5 % acetonitrile as diluent. To make the standard solutions, the following substances were applied: ATP (adenosine -5'-triphosphate disodium salt trihydrate, Roche diagnostics, cas:51963-61-2), ADP (adenosine-5'-triphosphate sodium salt, Sigma-Aldrich, cas: 20398-34-9), AMP (adenosine-5'-monophosphate sodium salt, Sigma aldrich, cas:149022-20-8), IMP (inosine-5'-monophosphate disodium salt hydrate, Sigma-Aldrich, cas:352195-40-5), In (inosine, Sigma-Aldrich, cas: 58-63-9), Hx (Hypoxanthin, Sigma-Aldrich, cas:68-94-0). The concentrations of ATP degradation products in the samples were calculated from μmol/L to μmol/g sample. ATP and IMP were both given as salts with a higher molecular weight than pure ATP and IMP. The

actual concentration was therefore recalculated and accounted for in the plotting.

3.6 Statistical analysis

One-way ANOVA and post-hoc multiple comparisons test were used to obtain all the statistical data. All results are given as mean value± S.D., with the exception of microbial counts, which are given as mean value± S.E.

Differences within the time of filleting were obtained by evaluating pre- and post-rigor filleted groups separately. Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c) in the plot, meanwhile significant differences between groups are indicated by different letters (X, Y, Z) in the chart legend.

Different numbers (1-2-3-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting.

Chapter 4

Results

The results of this study are summarised in the following sections. Groups are represented by different symbols and colors. Pre-rigor samples are illustrated with solid lines, meanwhile post-rigor samples are illustrated with dashed lines. This is schematically shown in Figure 4.1.

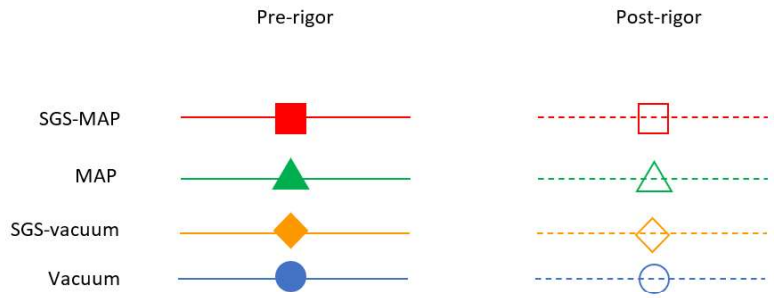


Figure 4.1: Overview of symbols, colors and lines used to indicate different groups in the plots of the following sections.

The data presented in this thesis are based on time elapsed after filleting. As post-rigor filleted samples were stored for 4 days during the resolution of rigor, age since slaughter differs between the pre- and post-rigor filleted groups. For pre-rigor filleted samples, days after slaughter and days after filleting are the same, meanwhile groups filleted post-rigor have a 4 days delay. For plots of ATP degradation compounds due to time after slaughter, see Appendix 6.5

4.1 Headspace gas composition and product pH

The headspace gas composition was analyzed in samples packed in modified atmospheres, i.e. MAP and SGS-MAP of both pre-rigor and post-rigor filleted salmon. Figure 4.2 illustrates the development of CO_2 (%) in headspace.

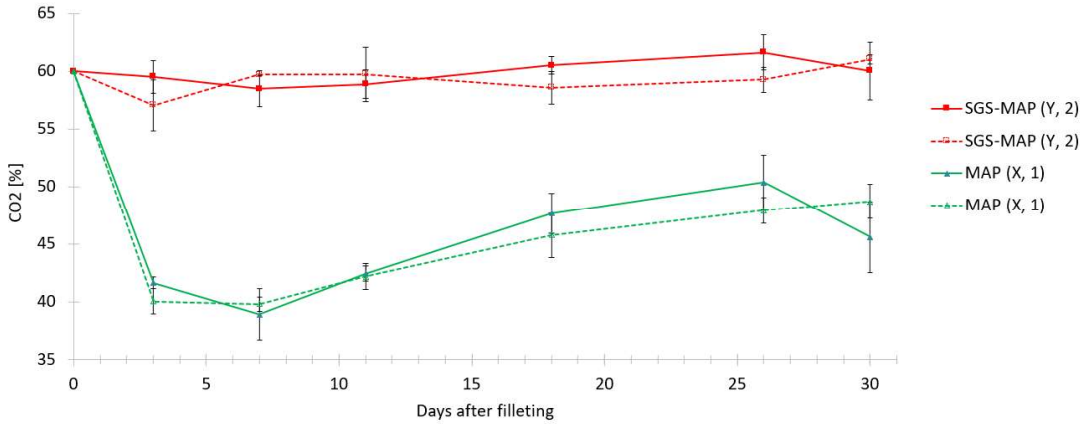
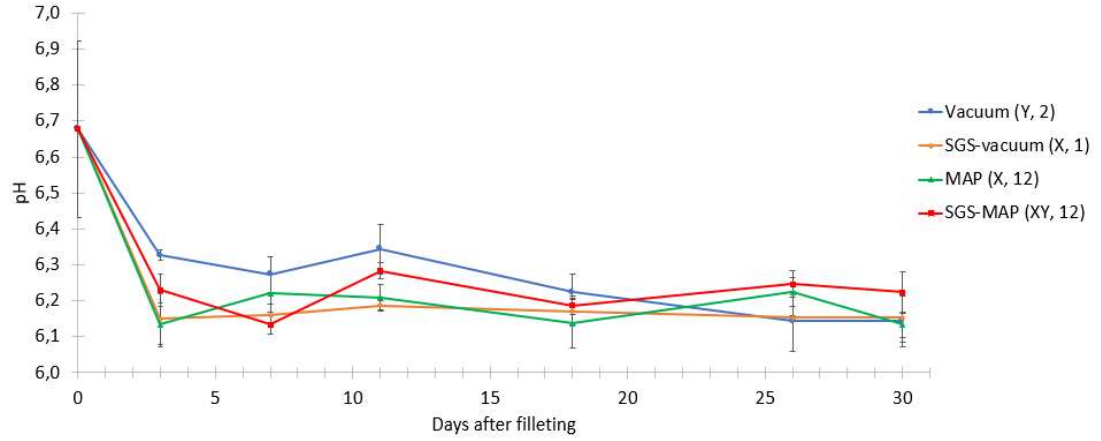


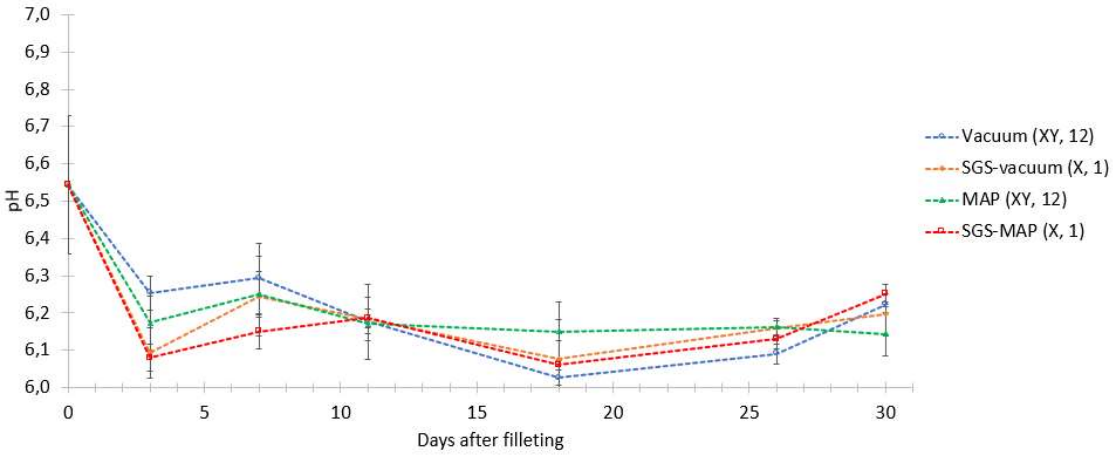
Figure 4.2: The percentage of CO_2 in headspace of modified atmosphere packed samples, given as means $\pm$ SD (*n=3*). Red color indicates SGS-treated samples, whilst green represents the controls. Pre-rigor filleted samples are indicated as SGS-MAP (■) and MAP (▲) with solid lines. Post-rigor filleted samples are indicated as (□) and MAP (△) with dashed lines. Significant differences ($p<0.05$) between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting.

SGS-MAP samples of both pre- and post-rigor filleting had a significantly higher ($p<0.01$) amount of CO_2 in headspace than pre- and post-rigor filleted MAP samples. SGS-MAP samples tended to stabilize just below the initial concentration, meanwhile MAP samples quickly dropped to around 40 % before gradually increasing. No significant differences were found between pre- and post-rigor filleted samples within the same processing method, i.e. between pre- and post-rigor filleted MAP samples and between pre- and post-rigor filleted SGS-MAP samples.

The muscle pH throughout the storage time is illustrated in Figure 4.3a for pre-rigor filleted samples and Figure 4.3b for post-rigor filleted.



(a)



(b)

Figure 4.3: Muscle pH in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. The pH data are mean values and the error bars represent S.D. (n=3).

At the first sampling, vacuum samples had the highest muscle pH in both pre- and post-rigor filleted samples, starting at 6.33 $\pm$ 0.02 and 6.25 $\pm$ 0.05, respectively. The pH of pre-rigor filleted vacuum samples generally remained higher compared to the other pre-rigor filleted groups and was significantly higher than pre-rigor filleted SGS-vacuum ($p=0.003$) and MAP samples ($p=0.021$).

Although no significant differences in pH were found among the post-rigor filleted groups based on the entire storage time, vacuum samples had a significantly higher pH than SGS-vacuum ($p=0.037$) and SGS-MAP samples ($p=0.025$) 3 days after filleting. The pH of post-rigor filleted vacuum samples quickly dropped 7 days after filleting and reached a pH minimum 18 days after filleting. At 26 days after filleting, vacuum samples had a significantly lower pH than MAP ($p=0.023$).

When considering the pH of both pre-rigor and post-rigor filleted samples, no significant differences were found between pre- and post-rigor filleted samples of the same processing and storage. Significant differences between pre- and post-rigor samples were only found between different processed groups, such as pre-rigor vacuum samples having a higher pH than pre-rigor SGS-vacuum (p=0.038), post-rigor SGS-vacuum (p=0.010), and post-rigor SGS-MAP (p=0.003). In this thesis, however, differences between pre and post-rigor filleted samples of the same processing method will be further discussed.

4.2 ATP degradation

The ATP degradation metabolites in both pre-rigor and post-rigor groups were analyzed 3, 7 and 11 days after filleting. ATP was not detected in the three samples of raw-material of neither pre-rigor nor post-rigor filleted salmon. However, small traces of ATP were found in some of the samples, with the highest amount being 0.6 ± 0.2 μmol/g. The concentrations of IMP and In of pre- and post-rigor filleted samples are given in Figure 4.4, 4.5a and 4.5b. The concentrations of Hx in pre-rigor filleted samples are given in Figure 4.6a and the post-rigor filleted concentrations are given in Figure 4.6b. The calculated K-values are given in Figure 4.7a and 4.7b. Meanwhile H-values are presented in Figure 4.8a for pre-rigor filleted samples and 4.8b for post-rigor filleted samples.

The concentrations of IMP in pre-rigor filleted and post-rigor filleted groups are illustrated in Figure 4.4.

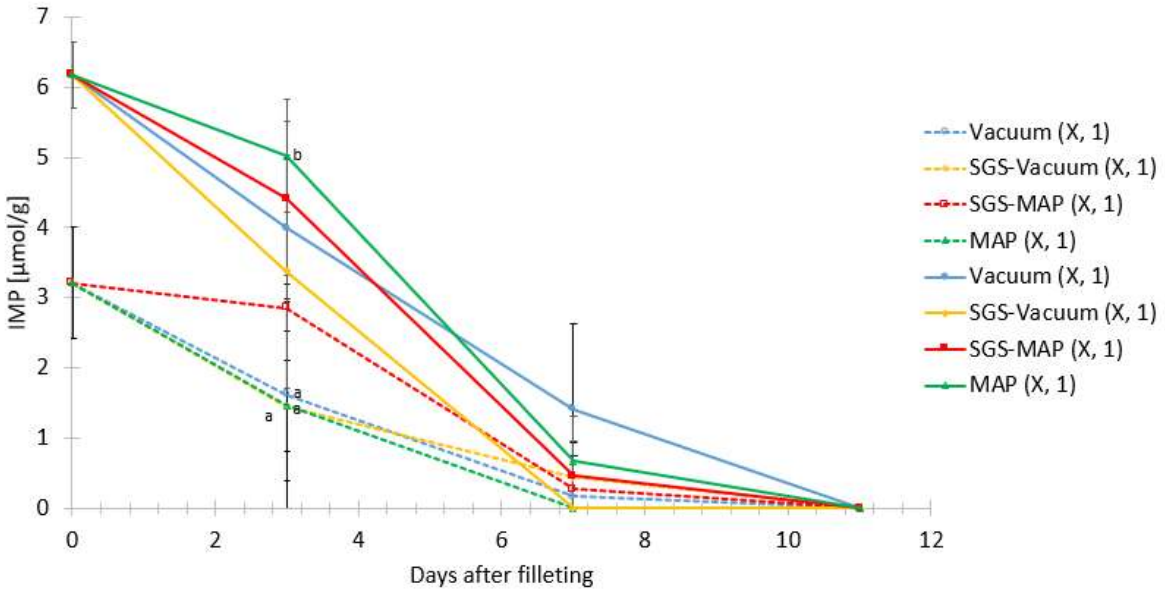
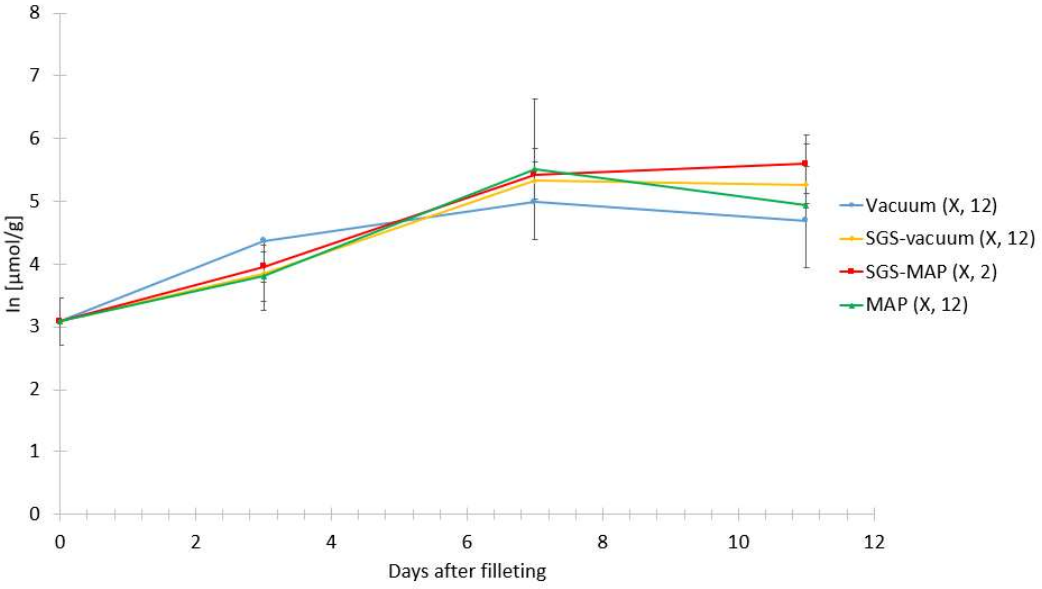


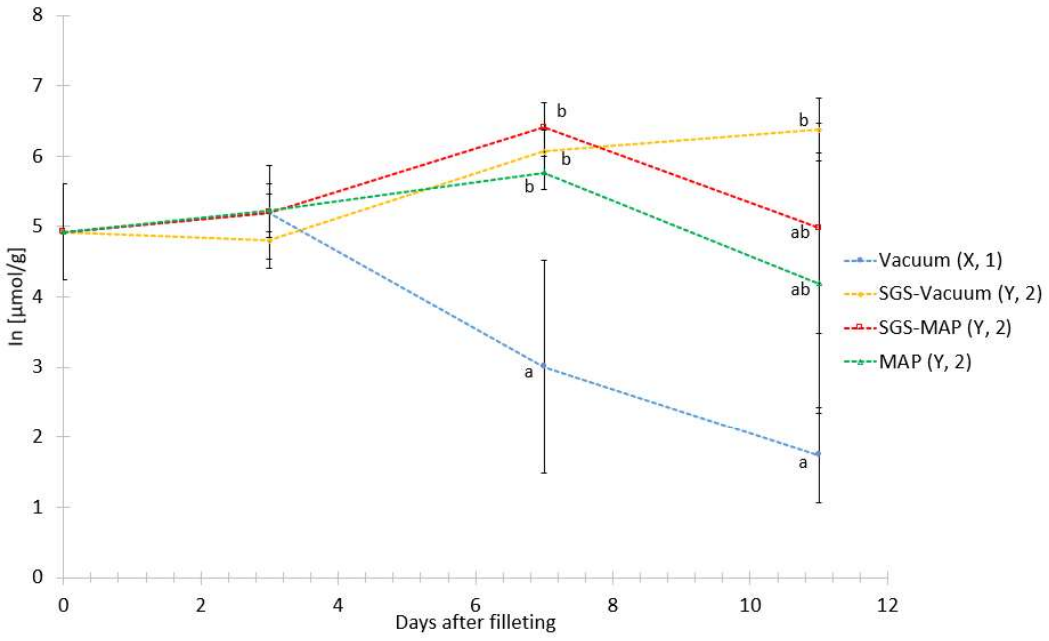
Figure 4.4: Concentration of IMP in samples filleted pre-rigor: vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■); and samples filleted post-rigor: vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. The data is presented as mean values and error bars represent S.D. (n=3).

The amount of IMP is decreasing in all groups throughout the storage time, as illustrated in Figure 4.4. The raw-material of pre-rigor filleted salmon had a significantly higher level of IMP present than the raw-material of post-rigor filleted salmon, which was measured 4 days post mortem ($p=0.005$). In general, pre-rigor filleted groups had higher amounts of IMP than their respective post-rigor filleted groups, when comparing days after filleting, although no significant differences were found when considering the entire storage time. Nevertheless, 3 days after filleting, pre-rigor filleted MAP samples were significantly higher than post-rigor filleted MAP samples ($p=0.020$), vacuum samples ($p=0.029$) and SGS-vacuum samples ($p=0.020$). At 7 days after filleting, nearly all the IMP had been degraded and no significant differences were evident. The highest amount of IMP was found in pre-rigor filleted vacuum samples, whereas IMP was not detected in neither pre-rigor filleted vacuum samples nor post-rigor filleted MAP samples. At the last sampling, 11 days after filleting, all the IMP had been degraded as nothing was detected in any groups.

The concentrations of Inosine in both pre-rigor and post-rigor filleted groups are illustrated in Figure 4.5.



(a)



(b)

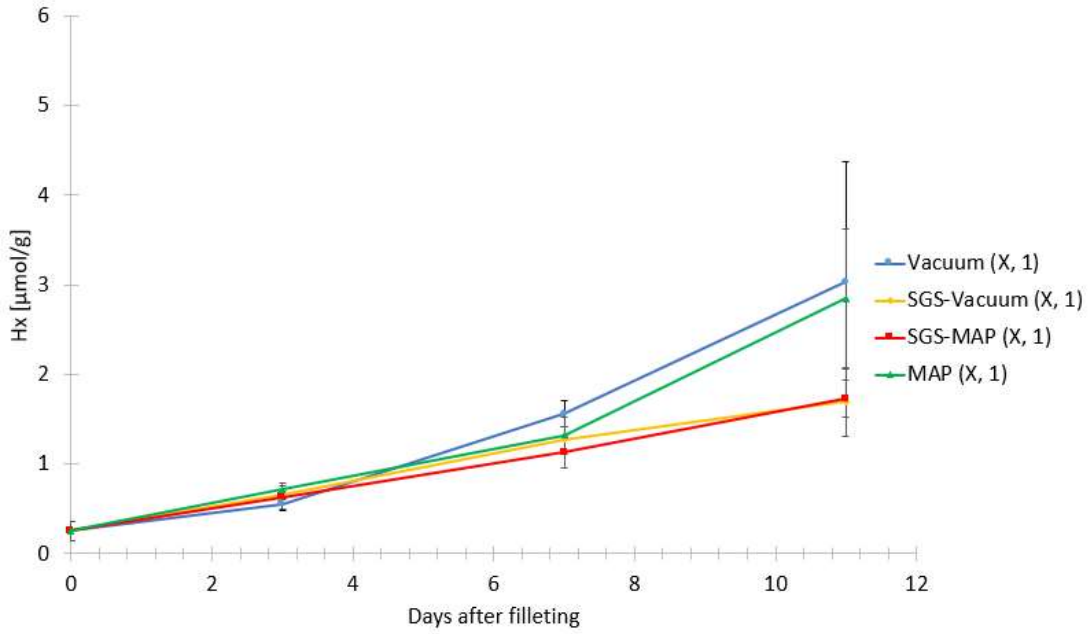
Figure 4.5: Concentration of inosine (In) in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. The error bars represent S.D. (n=3)

Raw-material of post-rigor filleted salmon had an inosine level of 4.9 $\pm$ 0.7 μmol/g (measured 4 days post mortem), which was significantly higher than the raw-material of pre-rigor filleted

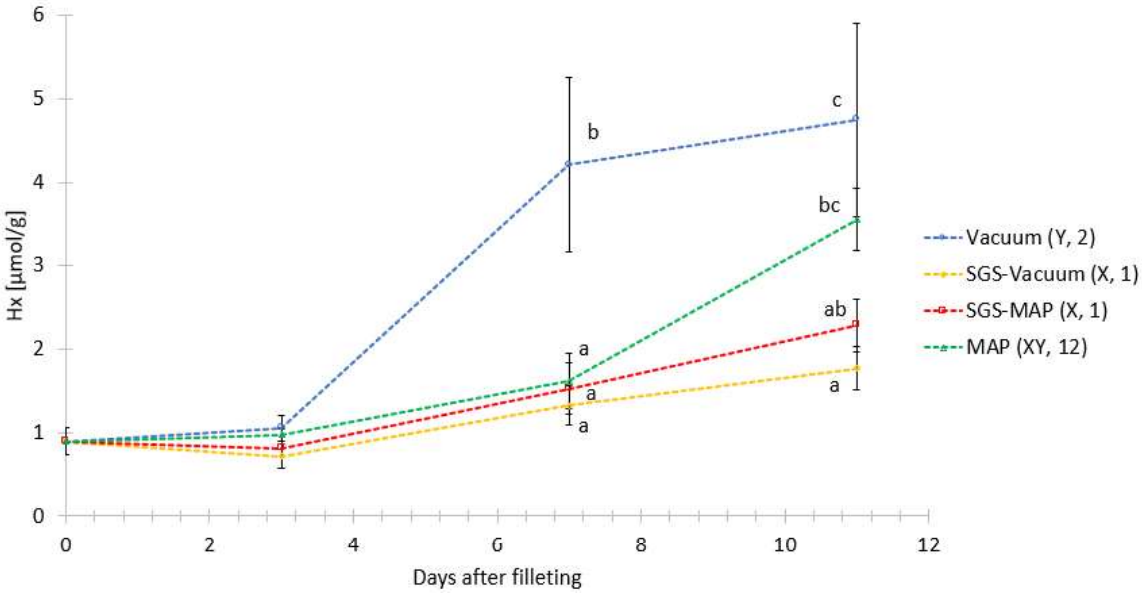
salmon of 3.1 $\pm$ 0.4 μmol/g. Comparing groups based on time of filleting, no significant differences were found between pre-rigor and post-rigor filleted groups of the same processing and storage.

All pre-rigor filleted groups, illustrated in Figure 4.5a, had a similar development, slightly increasing, and no significant differences were present. The post-rigor filleted groups started off with a relatively high amount of In, and post-rigor filleted SGS-vacuum samples were able to withhold the high level throughout storage, whereas vacuum samples quickly dropped. Post-rigor filleted vacuum was significantly lower than all the other post-rigor filleted groups ($p<0.05$).

The concentrations of Hypoxanthine (Hx) in pre-rigor and post-rigor filleted samples are shown in Figure 4.6a and Figure 4.6b, respectively.



(a)



(b)

Figure 4.6: Concentration of hypoxanthine (Hx) in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. The error bars represent S.D. (n=3)

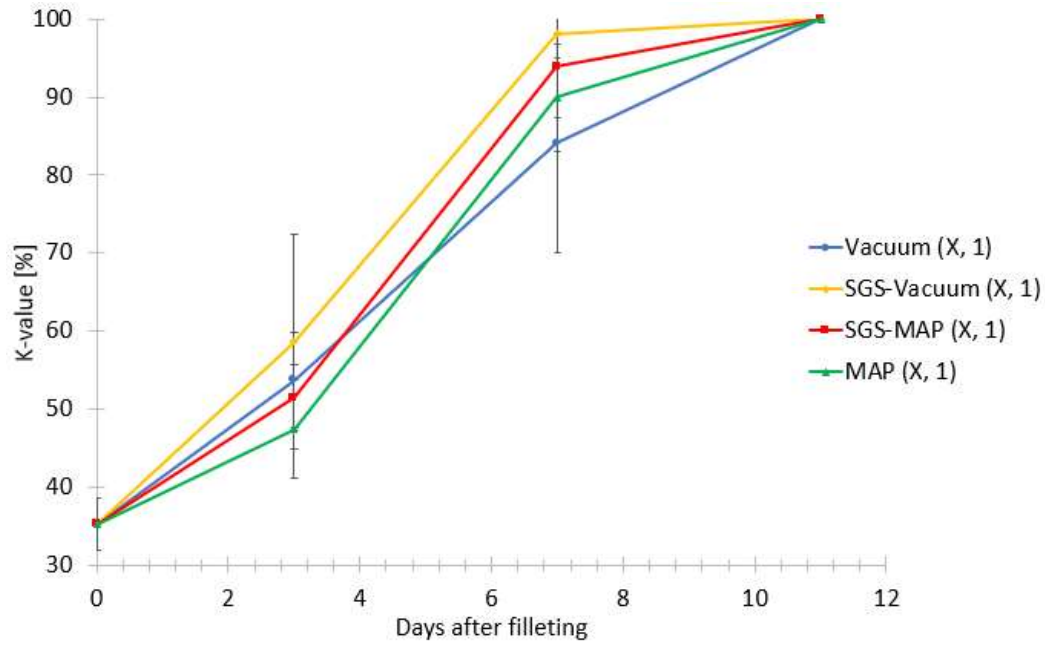
As expected, the amount of Hx is increasing with storage time for all pre-rigor and post-rigor filleted groups. When comparing pre- and post-rigor filleted samples, raw-material of post-rigor

filleted salmon (measured 4 days post mortem) had significantly higher amounts of Hx (p=0.005), and post-rigor filleted vacuum samples had a significantly higher Hx amount than pre-rigor filleted vacuum samples (p=0.002).

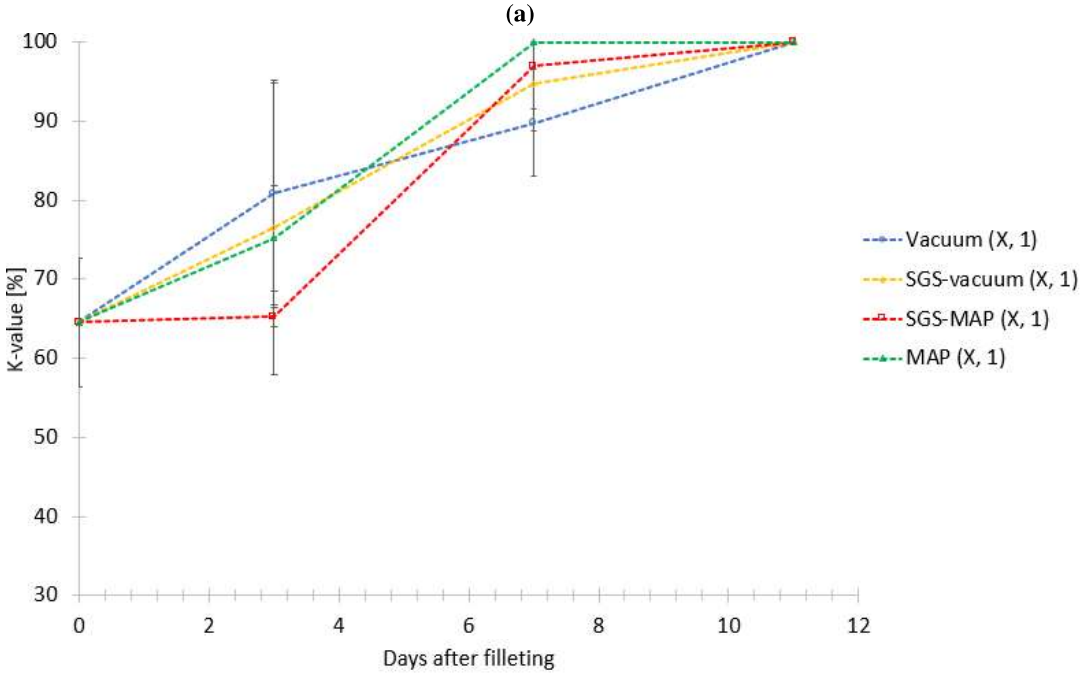
Considering the Hx-amounts in groups filleted pre-rigor (Figure 4.6a) all groups follow a similar trail from the beginning until 7 days after filleting. Between 7 and 11 days after filleting, the control groups, i.e. vacuum and MAP samples, began to increase more rapidly than the samples stored in SGS-MAP and SGS-vacuum. At 11 days after filleting, a gap had formed between the SGS-treated groups and the control groups. However, the gap was not of significant character.

The post-rigor filleted groups SGS-vacuum and SGS-MAP (Figure 4.6b) show similar tendencies as observed for the SGS treated pre-rigor filleted samples. The post-rigor filleted samples treated with SGS had relatively similar concentrations of Hx throughout the storage time, regardless of the packaging technique. At 3 days after filleting, the formation of Hx in vacuum samples started escalated and at 7 days after filleting they were significantly higher ($p<0.05$) than samples stored in SGS-vacuum, SGS-MAP and MAP. At the last sampling, SGS-vacuum samples were significantly lower than both vacuum (p= 0.002) and MAP samples (p=0.038). Lastly, SGS-MAP samples were significantly lower than vacuum samples (p=0.007).

K-values were obtained using equation 2.1 and plotted as a function of storage time. The K-value of pre-rigor and post-rigor filleted samples are given in Figure 4.7a and Figure 4.7b, respectively.



(a)



(b)

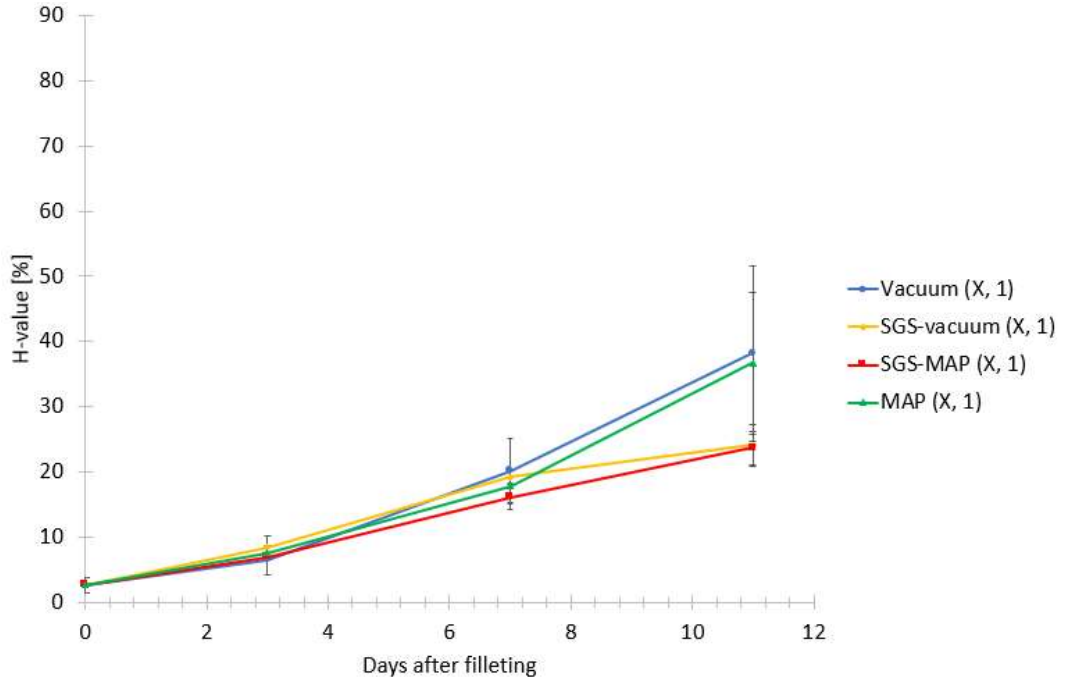
Figure 4.7: K-values of pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. The K-values are average values and the error bars represent S.D. (n=3)

The raw-material of pre-rigor filleted salmon had a K-value of 35 $\pm$ 3 %. The K-value was increasing in all groups until it reaches 100 % at the last sampling, 11 days after filleting. SGS-

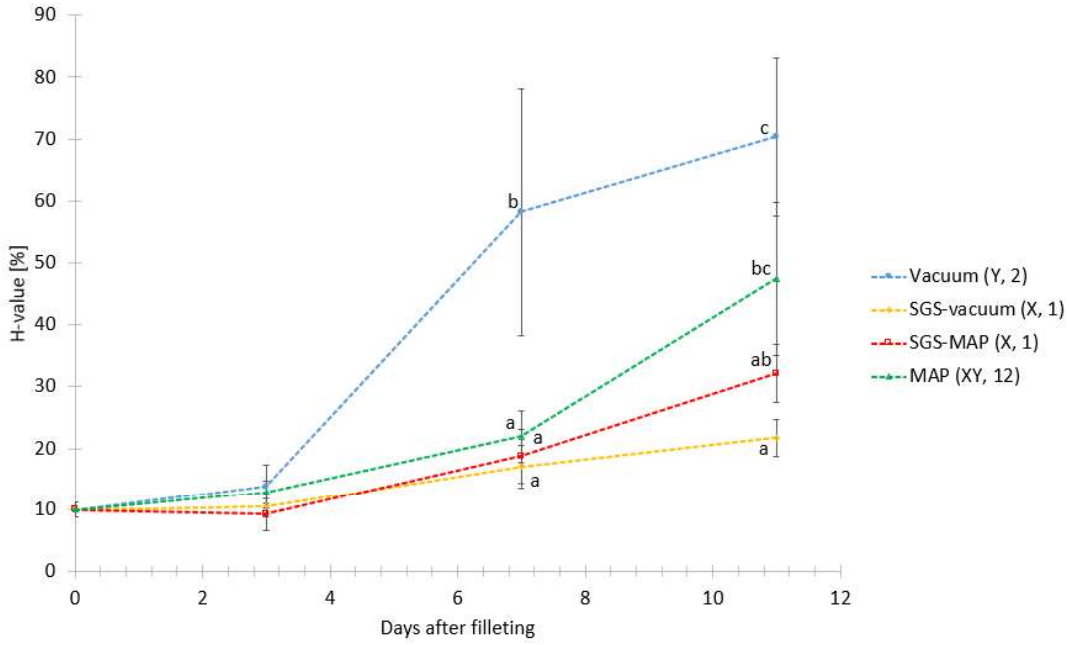
vacuum samples seemed to have the overall highest pre-rigor K-value, however, not significantly. The K-value of raw-material of post-rigor filleted salmon (measured 4 days post mortem) started at 65 $\pm$ 8 %, which was significantly higher than the raw-material of pre-rigor filleted salmon (p=0.005). The pre-and post-rigor filleted samples kept increasing until they reached 100% at 11 days after filleting, with the exception of pre-rigor MAP samples, which came to 100 % already at 7 days after filleting. Considering the post-rigor filleted groups; SGS-MAP samples tended to have a slightly lower K-value than MAP samples, however, no significant differences were present. Subsequently to the sampling 3 days after filleting, post-rigor filleted samples stored in MAP and SGS-MAP seemed to have a more rapid increase in K-value than the post-rigor filleted vacuum and SGS-vacuum samples.

When comparing the K-value between pre- and post-rigor filleted groups, no significant differences were evident based on the entire storage time nor on individual days.

H-values were obtained using equation 2.2 and plotted as a function of storage time. The H-value of pre-rigor and post-rigor samples are given in Figure 4.8a and Figure 4.8b, respectively.



(a)



(b)

Figure 4.8: H-values of pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The H-values were calculated using equation 2.2. Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. The H-values are presented as average values and the error bars represent S.D. (n=3)

Raw-material of pre-rigor filleted salmon had a H-value of 2.7 ± 1.1 %, meanwhile raw-material of post-rigor filleted salmon (measured 4 days post mortem) started significantly higher at 9.9 ± 1.2 % (p=0.002). At 3 and 7 days after filleting, all the pre-rigor filleted groups had nearly equal H-values. At 7 days after filleting, the pre-rigor filleted control groups began to increase more rapidly than pre-rigor filleted samples stored in SGS-MAP and SGS-vacuum. At the last sampling, 11 days after filleting, the groups had the following H-values; vacuum samples (38.2 ± 13.5 %), MAP samples (37 ± 11 %), SGS-vacuum samples (24.2 ± 3.2 %), and SGS-MAP samples (23.6 ± 2.6 %). However, the gap between SGS-processed groups and controls was statistically insignificant.

Figure 4.8b provides the post-rigor concentrations and differences between groups appear more prominent compared to the pre-rigor groups. Based on the entire storage time, H-values of post-rigor filleted vacuum samples were significantly higher than SGS-vacuum samples (p=0.004) and SGS-MAP samples (p=0.012). The first days after filleting, i.e. until 3 days after filleting, all post-rigor filleted groups had a more or less similar H-value. Subsequently, samples stored in vacuum escalated and obtain an average H-value of 58 ± 20 % at 7 days after filleting. The remaining groups had a slower development, thus being significantly lower than the post-rigor filleted vacuum samples (p<0.05). At the last sampling, 11 days after filleting, the post-rigor filleted groups became more disperse with vacuum samples having the highest average H-value of 70 ± 13 %. Post-rigor filleted vacuum were significantly higher than SGS-MAP (p=0.004) and SGS-vacuum (p=0.001). Additionally, one should note that post-rigor filleted SGS-vacuum samples were significantly lower than post-rigor filleted MAP samples(p=0.038).

Finally, when comparing H-values based on time of filleting, no significant differences were present between SGS-processed groups of equal storage conditions. The only significant difference in H-value due to time of filleting was in the vacuum control group, where post-rigor filleted vacuum samples were significantly higher than pre-rigor filleted (p<0.01). However, significant differences between control groups due to time of filleting will not be further discussed.

4.3 Microbial results

The growth of APC was registered over a period of 30 days after filleting. The microbial counts are presented as means ± SE. The total counts obtained from the four pre-rigor filleted groups and the four post-rigor filleted groups are presented in Figure 4.9a and Figure 4.9b, respectively.

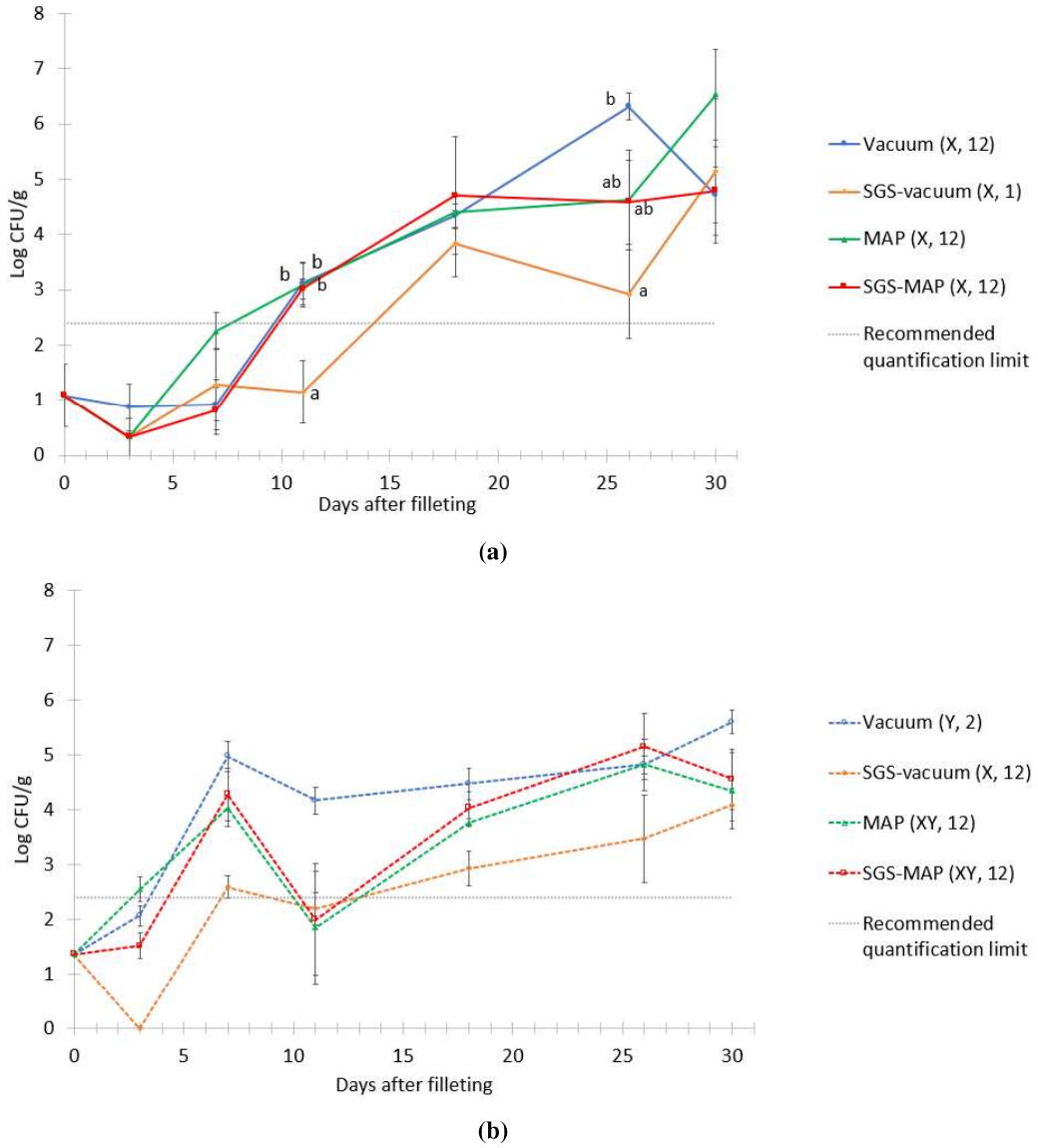


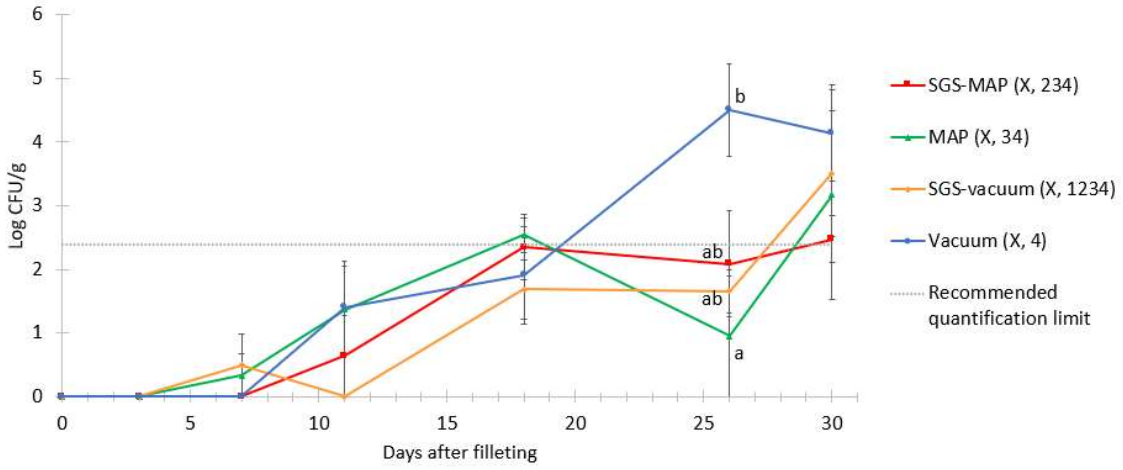
Figure 4.9: The average aerobic plate count (APC) in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The grey, dotted horizontal line represent the recommended quantification limit of log(250) defined by NMKL [113]. Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. Error bars represent S.E. (n=3)

The raw-material of pre-rigor filleted salmon had a bacterial concentration of 1.1 ± 0.6 log CFU/g, well below the recommended quantification limit of the method. None of the pre-rigor filleted groups exceeded the recommended quantification limit at the first two samplings, i.e. 3 and 7 days after filleting. Pre-rigor filleted SGS-vacuum samples were significantly ($p<0.05$) lower than the other pre-rigor filleted groups at 11 days after filleting and significantly lower than pre-rigor filleted vacuum samples at 26 days after filleting ($p=0.043$).

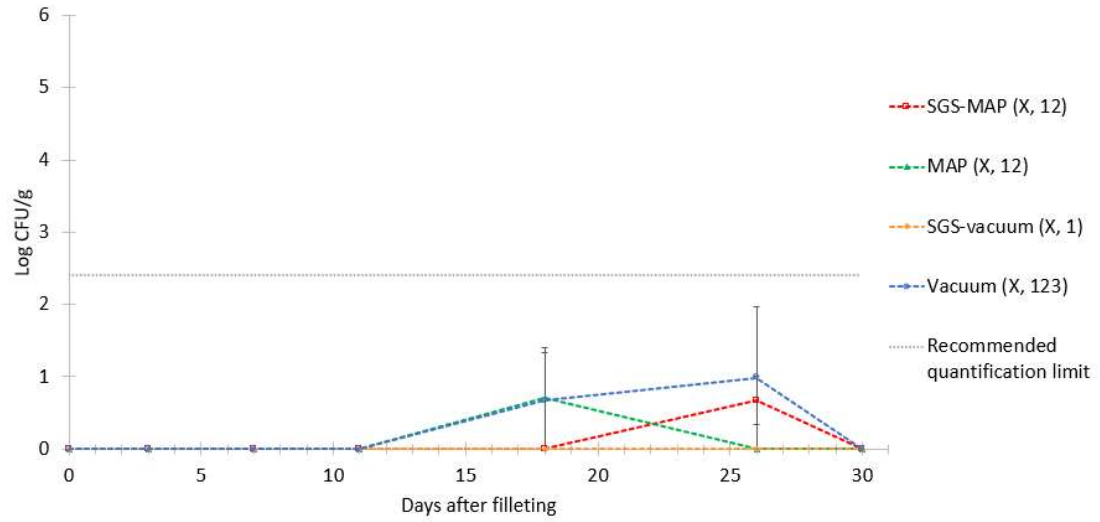
The aerobic bacterial concentration of the raw-material of post-rigor filleted salmon (measured 4 days post mortem) was 1,36 $\pm$ 0,06 log CFU/g. All four post-rigor filleted groups exceeded the recommended quantification limit 7 days after filleting. The growth of post-rigor filleted MAP and SGS-MAP samples followed a similar trail with similar values throughout the storage time. The growth in the two vacuum packed groups developed in a similar pattern as well, however, at two different levels. Post-rigor filleted SGS-vacuum samples had significantly lower counts than vacuum stored samples ($p=0.002$). Additionally, post-rigor filleted SGS-vacuum samples tended to have lower growth than post-rigor filleted MAP and SGS-MAP samples, though not significantly ($p>0.128$).

Comparing the APC of pre- and post-rigor filleted samples, the only significant difference was pre-rigor SGS-vacuum samples being significantly lower than post-rigor vacuum samples ($p=0.039$).

The growth of the H_2S-producing bacteria is illustrated in Figure 4.10a for pre-rigor filleted samples and in Figure 4.10b for post-rigor filleted samples.



(a)



(b)

Figure 4.10: The average counts of H_2S-producing bacteria in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The grey, dotted horizontal line represent the recommended quantification limit of log(250) defined by NMKL [113]. Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. Error bars represent S.E. (n=3)

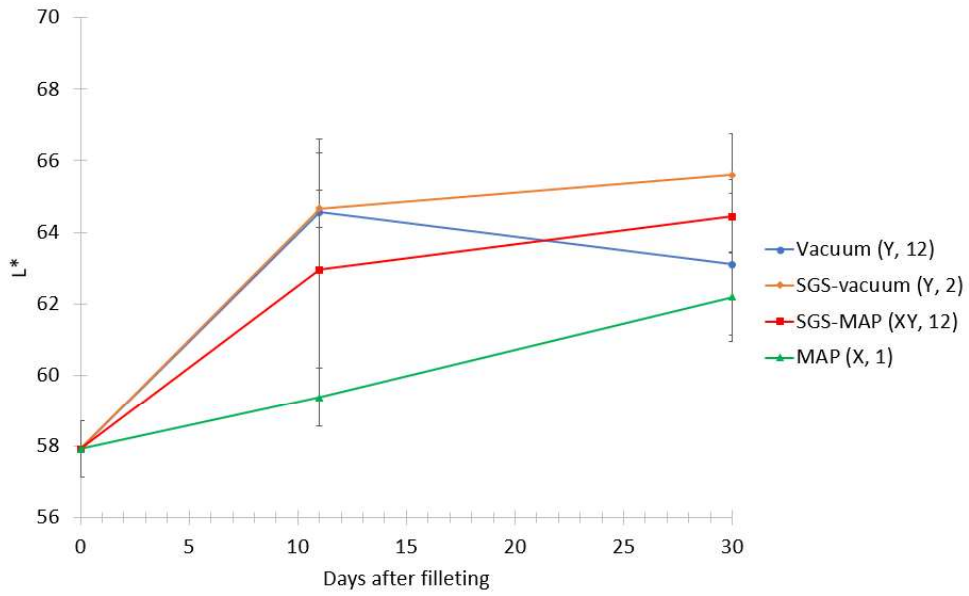
At 7 days after filleting, the first detectable growth of H_2S-producing bacteria in pre-rigor filleted samples was found in MAP and SGS-vacuum samples. At 18 days after filleting, all four pre-rigor filleted groups had growth. However, they were not quantifiable, as recommended by the method, until the last sampling, 30 days after filleting, with the exception of pre-rigor filleted vacuum, which exceeded the recommended quantification limit 26 days after filleting. Vacuum rose to a relatively high point of 4.5 log CFU/g 26 days after filleting, though only significantly higher than MAP samples ($p=0.039$).

Growth of H_2S-producing bacteria in post-rigor filleted samples was not detected until day 18 and was found in both of the control groups. No further growth was detected in the MAP group, meanwhile vacuum had growth at the next sampling, 26 days after filleting. Additionally, H_2S-producing bacteria was detected in the SGS-MAP group as well on sampling day 26. Lastly, no growth was found in the SGS-vacuum samples. The average growth in all four groups was below 1 log CFU/g.

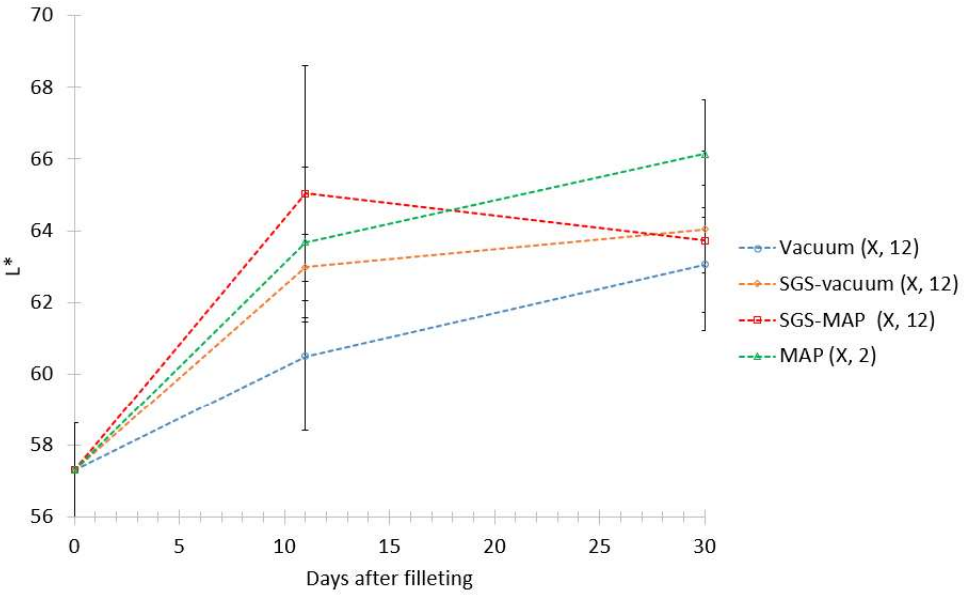
Significant differences in H_2S producing bacteria counts between pre- and post-rigor filleted salmon were only found between the control groups; pre-rigor filleted vacuum samples were significantly higher than post-rigor filleted vacuum samples (p=0.001), and pre-rigor filleted MAP samples were significantly higher than post-rigor filleted MAP samples (p=0.043). No significant differences were found between pre- and post-rigor filleted SGS-treated salmon, i.e. between pre- and post-rigor filleted SGS-MAP (p=0.100) and between pre- and post-rigor filleted SGS-vacuum (p=0.062).

4.4 Color

The fillet lightness (L*) of pre and post-rigor filleted samples are illustrated in Figure 4.11a and Figure 4.11b, respectively. Colour measurements of the fillet lightness (L*) showed that time of filleting did not have any significant affected on neither raw-material nor groups, with the exception of post-rigor filleted MAP samples being significantly lighter than pre-rigor filleted MAP samples (p=0.013). Table values of the color characteristics in pre- and post-rigor filleted samples are given in Appendix A.3 and A.4, respectively.



(a)



(b)

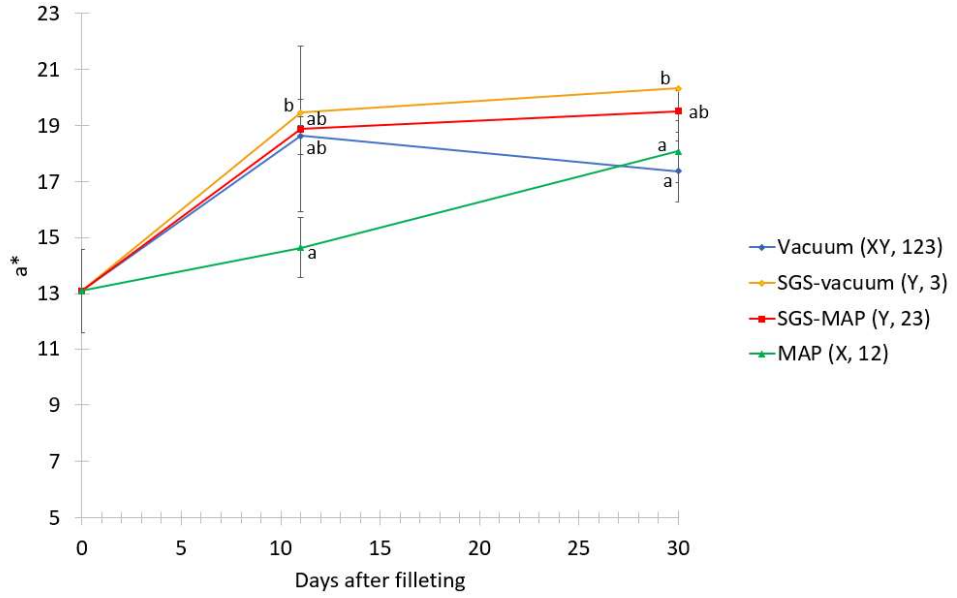
Figure 4.11: The lightness (L*) in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. Error bars represent S.E. (n=3)

All the pre-rigor groups showed increased lightness (L*) compared to the raw-material. At 11 days after filleting, pre-rigor filleted vacuum and SGS-vacuum samples had approximately the same score, which was also the highest lightness values, meanwhile MAP samples had the lowest and the increase from raw-material was insignificant. SGS-treated samples tended to be lighter

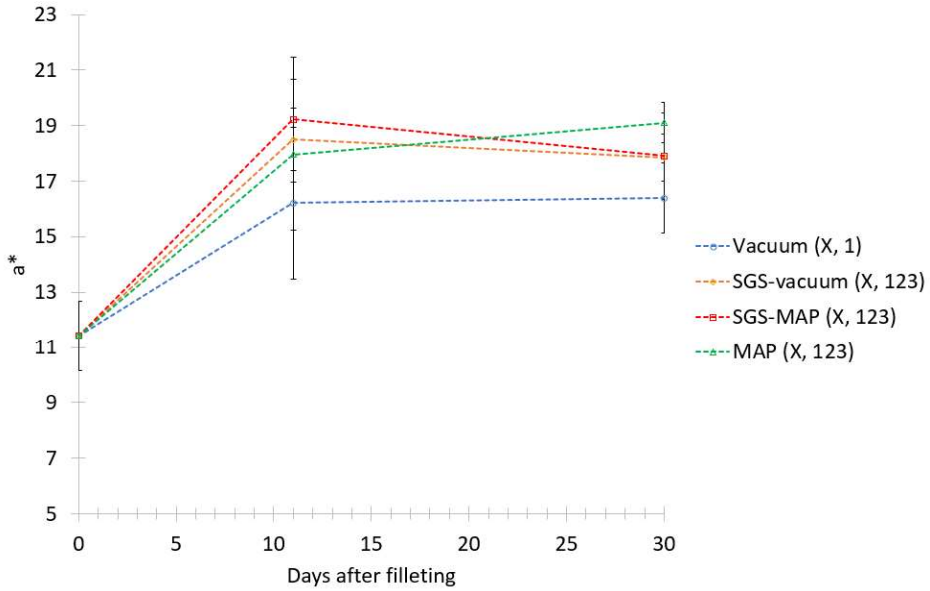
than the non-SGS treated samples, especially in the MAP groups, and although pre-rigor MAP samples had a 3 points lower average L* value compared to pre-rigor SGS-MAP, the difference was not significant. However, there was a significant correlation of 0.628 between CO_2 in headspace and L-value, indicating increasing lightness with increasing concentrations of CO_2. Lastly, pre-rigor filleted MAP samples were significantly lower than pre-rigor filleted vacuum ($p=0.048$) and pre-rigor SGS-vacuum samples ($p=0.003$).

As in the case of pre-rigor filleted groups, the post-rigor filleted groups significantly increased ($p<0.05$) in lightness compared to raw-material (measured 4 days post mortem). Based on the sampling at 11 days after filleting, all SGS-treated groups were situated above their respective control groups. In the last sampling, though, SGS-MAP samples were situated below the MAP samples. However, no significant differences were found between any of the post-rigor groups in terms of lightness.

The fillet redness (a*), is illustrated in Figure 4.12a for pre-rigor filleted groups and 4.12b for post-rigor filleted groups. The fillet redness did not get affected by the time of filleting as no significant differences were present between pre-rigor and post-rigor of neither raw-material nor groups.



(a)



(b)

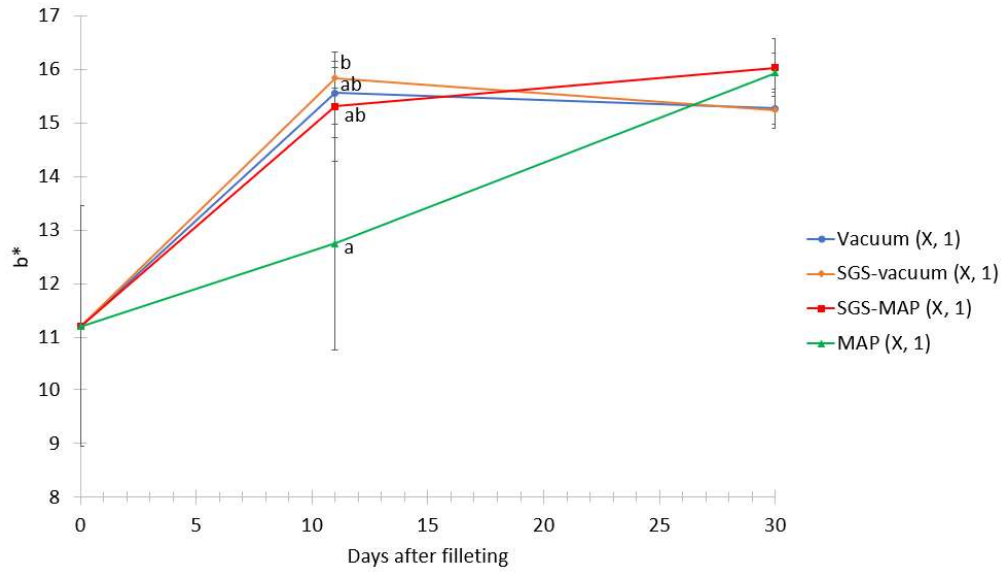
Figure 4.12: The redness (a*) in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. Error bars represent S.E. (n=3)

Considering the pre-rigor filleted groups, they all had a significant increase in fillet redness (a*) compared to the raw-material. Furthermore, pre-rigor filleted SGS-MAP, vacuum and SGS-vacuum samples had a more rapid increase in redness than pre-rigor filleted MAP samples. Ad-

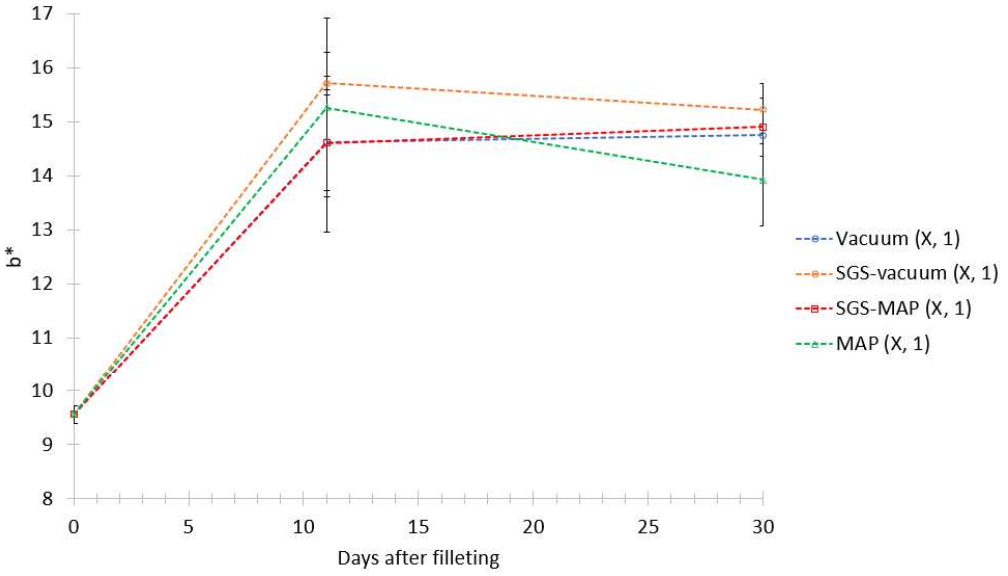
ditionally, the pre-rigor filleted SGS-vacuum samples were significantly higher than the MAP samples (p=0.027). At the last sampling, both the SGS-treated groups had higher scores than the control groups, with SGS-vacuum being significantly higher than both vacuum (p=0.013) and MAP samples (p=0.049). Lastly, based on the entire storage time, MAP samples had a significantly lower fillet redness (a*) than SGS-MAP samples (p=0.024), as well as SGS-vacuum samples (p=0.004).

The post-rigor filleted groups also had a rapid increase of fillet redness (a*), where vacuum with the smallest increase was almost 5 scores higher than the raw-material. Thus, all groups had a significant increase in fillet redness (a*) compared to the raw-material (measured 4 days post mortem) ($p<0.05$). The fillet redness stabilized and no major differences were found between 11 and 30 days after filleting. The SGS-treated vacuum group also tended to have a higher score than the control vacuum group, whereas SGS-MAP samples had fairly similar scores as MAP samples, however, no significant differences were present.

The b* values of pre- and post-rigor samples are given in Figure 4.13a and 4.13b, respectively.



(a)



(b)

Figure 4.13: The yellowness (b*) in pre-rigor (a) and post-rigor (b) filleted salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). Significant differences ($p<0.05$) on specific days are indicated with different letters (a, b, c), meanwhile significant differences between groups within the same time of filleting are indicated by different letters (X, Y, Z) in the chart legend. Different numbers (1-4) in the chart legend indicated significant differences ($p<0.05$) between groups due to time of filleting. Error bars represent S.E. (n=3)

All pre-rigor filleted groups ha a significant increase in fillet yellowness (b*) 11 days after filleting compared to the raw-material. Pre-rigor filleted SGS-vacuum, vacuum and SGS-MAP samples had a similar fillet yellowness (b*) and were notably higher than MAP samples, though only pre-rigor filleted SGS-vacuum samples were significantly higher than pre-rigor filleted MAP samples.

MAP samples continued to increase the following sampling, whereas the other pre-rigor filleted groups had minor changes. At the end of storage, all groups had fairly equal intensity in yellowness (b*). Furthermore, no significant differences were found between pre-rigor filleted groups when taking the entire storage period into account.

The fillet yellowness (b*) of post-rigor filleted samples had similar development as the pre-rigor filleted groups, a rapid increase before stabilizing. In contrast to the development of the pre-rigor filleted groups, post-rigor filleted MAP samples were relatively equal to the rest of the groups. No significant differences were present between any of the post-rigor filleted groups.

Chapter 5

Discussion

5.1 Headspace gas composition and product pH

As seen in Figure 4.2, time of filleting did not have any significant influence ($p>0.938$) on the headspace gas composition of MA-packages during storage, consequently demonstrating that pre-rigor filleting is an equally suitable approach as post-rigor filleting in terms of headspace gas composition. The processing and packaging method, on the other hand, i.e. SGS-MAP versus MAP, had a significant impact ($p<0.05$). By saturating the salmon samples with carbon dioxide prior to packing, the headspace gas composition was maintained at a high level throughout the storage time. This is an interesting finding, and it could be hypothesized that the SGS processed salmon will achieve a longer shelf-life than the control, as seen in the case of MA packaged Ready-to-Eat shrimps [8] and skinless chicken [10] where the APC was significantly reduced by the SGS-treatment.

It appears that pre- and post-rigor filleted SGS-MAP samples had a rather stable amount of CO_2 in headspace, whereas pre- and post-rigor filleted MAP samples had an increase 7 days after filleting. Both SGS-MAP and MAP samples had similar APC counts, implying that any microbial production of CO_2 should be similar. However, one can question if the composition of the microbiota has been affected by the SGS-treatment. Lerfall et al. (2008) reported the relative abundance of the dominating bacterial taxa in saithe filets under different conditions where *Photobacterium spp.* was reduced from 90 to 80 % and *Shewanella spp.* increased from 10 to 20 % when changing the headspace gas composition from high CO_2/low N_2 to low CO_2/high N_2. Furthermore, a shift of the dominant microbiota occurred (from *Pseudomonas sp.* to *Photobacterium phosphoreum* and *Lactococcus piscium*) after 7 to 10 days of storage in MA-packages was reported by Macé *et al.* (2013). However, if the shift is dependent on the CO_2 concentration in headspace and/or cause production of CO_2 was not investigated.

Based on observations, SGS-MAP samples of pre- and post-rigor had a greater tendency of bulking compared to MAP (see Apendix 6.3). The bulking may indicate that the pre-dissolved CO_2 in the tissue of SGS-MAP samples diffused out. If the product has a higher percentage of dissolved CO_2 than the headspace percentage, then the pre-dissolved CO_2 will diffuse out of the tissue ac-

cording to the law of equilibrium. However, as equilibrium has been reported to occur within 3 days after packaging [114, 115], this does not appear to be the case. Another possible explanation is the atmospheric pressure. If CO_2 has diffused out of the product and into the headspace, the gas diffusion is in accordance to Henrys law [114, 116, 117]. The concentration of dissolved CO_2 is dependent on the partial pressure of CO_2 and Henrys constant for CO_2. MAP packages are semi-rigid, hence fluctuations of the atmospheric pressure affect the amount of dissolved CO_2. Rotabakk *et al.* (2007) reported that a difference of 10% in the atmospheric pressure results in a 100 ppm difference in the amount of solubilized CO_2. The atmospheric pressure during storage is given in Appendix 6.3, and bulking appeared when greater fluctuations in air pressure occurred. Lastly, the rate of diffusion is also temperature-dependent according to Henrys constant and the solubility of CO_2 is inversely proportional to temperature [117], hence increased temperatures may also cause bulking. Unfortunately, the storage temperature was not monitored between the samplings.

Considering the product pH given in Figure 4.3, time of filleting did not cause any significant differences between groups of equal processing and packaging, for instance, between pre- and post-rigor filleted SGS-MAP (p=0.070). The SGS-treatment, on the other hand, caused significantly lower pH in pre-rigor filleted SGS-vacuum samples compared to pre-rigor filleted vacuum samples. As solubilized CO_2 has an acidic effect, explained by equation 2.3, the low pH may imply high amounts of dissolved carbon dioxide in the muscle. Consequently, the microbial growth is restrained by the presence of CO_2 both directly, as the gas is believed to disrupt metabolisms in the microbial cells [79], and indirectly by the acidic environment. Nevertheless, this outcome is contrary to that of Rotabakk *et al.* (2008), who found pH to be unaffected by the SGS-processing [9].

Comparing MAP and SGS-MAP samples, one would assume a lower pH in SGS-MAP samples as they had significantly higher amounts of carbon dioxide present in headspace. Rotabakk *et al.* (2006) also reported a drop in pH of 0.15 units of fillets under CO_2 atmospheres [10]. However, no drop in pH of SGS-processed MAP samples was observed, and they were not significantly lower than MAP samples. Given the results, it could be debated if samples stored in MAP and SGS-MAP had the same amount of dissolved CO_2. Unfortunately, it is impossible to determine as the amount of solubilized CO_2 in the muscle of the samples was not measured.

5.2 ATP degradation

IMP

A high IMP level is desirable as it is positively correlated to the perception of freshness and linked to the umami taste [39]. IMP is generated by the action of endogenous enzymes, and its depletion into inosine is mainly caused by the activity of endogenous enzymes.

IMP levels due to time of filleting[1]

Although pre-rigor filleted samples initially had a significantly higher IMP level than post-rigor filleted samples, they had more or less the same amount of IMP 7 days after filleting. The results indicate a more rapid decrease of IMP in pre-rigor filleted samples. When considering the IMP amounts in post-rigor filleted groups, they appear to have a more steady decrease, especially since they were stored as HOG in four days before monitoring took place. Based on the initial IMP levels, the autolytic changes in pre-rigor samples have not evolved as much as in the post-rigor filleted samples. However, the following rapid decrease in pre-rigor samples is not abnormal as early filleting may accelerate the autolytic processes due to rough handling or crushing, which stimulates nucleotide breakdown [45].

One could debate if days after slaughter is more appropriate to use in terms of comparing IMP levels as the autolytical processes take place immediately after slaughter. When comparing IMP due to time of slaughter (see Appendix 6.5), only two sampling points from days after filleting and days after slaughter are overlapping. Day 3 and 7 after filleting of post-rigor salmon equals to 7 and 11 days after slaughter, respectively. Hence, post-rigor sampling 3 and 7 days after filleting can be directly compared to post-rigor sampling day 7 and 11 after slaughter. Based on these two samplings, post-rigor filleted groups generally had a higher IMP level than pre-rigor filleted groups, with the exception of vacuum samples, which had approximately the same amount. However, only post-rigor filleted SGS-MAP samples were significantly higher than pre-rigor filleted SGS-MAP samples ($p=0.027$).

Nevertheless, due to the resolution of rigor prior to filleting, post-rigor filleted fish are not available for the consumer the first days after slaughter, in contrast to pre-rigor filleted fish. Even though the autolytical processes seem to be more rapid in pre-rigor filleted salmon, they are available 4 days before post-rigor filleted salmon. Hence, the IMP level based on days after filleting seems more relevant from a consumers' perspective as the fish is accessible from this point. Interestingly, a depleted IMP level seems to occur at a relatively similar time, 7 days after filleting, for both pre-rigor and post-rigor. Even though the autolytical processes seem to be more rapid when filleting pre-rigor, the pre-rigor filleted still have a higher level of IMP the first days after filleting. Whether pre-rigor or post-rigor should be applied, in terms of autolytical processes, seems to have a small impact on the final product.

IMP levels due to SGS-processing

In order to determine the impact of SGS-processing, more frequent samplings should have been conducted the first days after storage. The IMP level was almost depleted in all the pre- and post-rigor filleted groups by day 7 after filleting. Thus, there is only one sampling (3 days after filleting), which indicates how SGS may have affected the IMP levels. If the arrangement of groups at 3 days after filleting is representative for the entire interval is not possible to determine. Furthermore, in sufficiently small sample volumes of biological material, it is reasonable to expect large deviations. However, given that sampling day 3 is more or less characteristic for

[1] IMP, In, Hx, H- and K-values in pre- and post-rigor filleted samples due to time of slaughter is given in Appendix 6.5.

the arrangement, no impact of the SGS-processing seemed to be present. In the pre-rigor filleted samples, SGS-treated groups had lower IMP levels than their respective control groups. All the post-rigor filleted groups had more or less equal amounts of IMP, with the exception of SGS-MAP, which had a notably higher level than the rest. However, post-rigor filleted SGS-MAP samples were not significantly higher than post-rigor filleted MAP samples. As the amount of IMP is mainly influenced by the activity of endogenous enzymes, which was also confirmed by the non-existing correlation between APC/H_2S producing bacteria counts and IMP, it seems as if the SGS-treatment did not affect the activity of endogenous enzymes.

Inosine

The inosine concentration is a combination of formation from IMP, mainly catalyzed by endogenous enzyme activity, and conversion into Hx, mainly catalyzed by microbial enzymes. When the level of inosine is increasing, more inosine is formed from IMP than what is converted into Hx. This trend is typical during the early stages of storage when the microbial activity is still low. Figure 4.5a illustrates this as the inosine amount steadily rose for all pre-rigor groups until 7 days after filleting before leveling off. When the microbial growth accelerates, consequently increasing the microbial enzyme activity, the inosine level will eventually stabilize until further conversion into Hx results in a decline. As a decline is mainly linked to a notably high microbial enzyme activity, it is an undesirable development.

Inosine levels due to time of filleting

In brief, it appears that pre-rigor filleted samples started increasing from 3 μmol/g, before stabilizing at 5 μmol/g, whereas post-rigor filleted groups, with the exception of SGS-vacuum samples, began at a higher, stabilized level of 5 μmol/g before decreasing.

The decrease occurred in post-rigor filleted vacuum samples 3 days after filleting and 7 days after filleting for post-rigor filleted SGS-MAP and MAP samples. This can partially be explained by the high APC values 7 days after filleting of post-rigor filleted samples. However, post-rigor filleted MAP and SGS-MAP samples had a drop in the APC 11 days after filleting, resulting in approximately equal counts as the pre-rigor filleted groups. The post-rigor filleted SGS-vacuum samples, on the other hand, were able to maintain the initial In level. Furthermore, it is important to emphasize that post-rigor filleted samples were stored as HOG for 4 days before filleting and processing, thus the degradation caused by endogenous enzymes had been ongoing for several days, during the resolution of rigor, before sampling took place. Consequently, the raw-material of post-rigor filleted salmon had significantly higher levels of In than pre-rigor filleted. This was also confirmed by the IMP level in the raw-material, where post-rigor filleted salmon had significantly lower amounts than pre-rigor filleted raw-material, indicating that the autolytical degradation, i.e. converting IMP into In, was more evolved in the post-rigor filleted samples.

Although no significant differences were present between pre- and post-rigor filleted groups of equal processing and packaging pre-rigor seems to be more suitable as no marked decline was observed.

Inosine levels due to SGS-processing

The impact of SGS-processing on the inosine level was most prominent between the vacuum packed groups. Post-rigor filleted vacuum samples had a rapid inosine fall 3 days after filleting, which also corresponds to the increase in APC. Although post-rigor filleted SGS-vacuum samples also exceeded the APC quantification limit 3 days after filleting, it continued to have increasing inosine amounts, resulting in a significantly higher level of inosine. As microbial enzymes are mainly responsible for converting In into Hx, the trend indicates that the SGS-treatment restrained the microbial enzyme activity in the post-rigor filleted vacuum packed groups.

A markedly decrease in In level, followed by a rapid increase in Hx is undesirable as it implies high microbial enzyme activity and accumulation of the undesirable Hx compound. This is observed in post-rigor filleted vacuum samples where the inosine amount decreased, and the Hx amount had a considerable increase 3 days after filleting and onwards. Hence, it is clear that the microbial enzymes in post-rigor filleted vacuum samples were rapidly converting inosine into Hx. In comparison, the SGS treated post-rigor vacuum samples had a gradual increase of In and the amount of Hx was low. The differences between In and Hx levels in post-rigor filleted vacuum and SGS-vacuum samples demonstrate a clear advantage of applying SGS. However, SGS had no apparent effect on the amount of In in pre-rigor filleted samples compared to the controls. The reason remains unclear but could be due to the 4 days delay of post-rigor filleted samples, resulting in more microbial enzyme activity, consequently implying that the small conversion of In into Hx in pre-rigor filleted groups is mainly caused by endogenous enzymes, which have been assumed unaffected by SGS. It would have been interesting to see the In levels of pre-rigor filleted samples 15 days after filleting, which would have been equal to post-rigor filleted samples 11 days after filleting when counting as days after slaughter to see if a decline appeared.

In contrast to post-rigor filleted SGS-vacuum and vacuum samples, post-rigor filleted SGS-MAP and MAP samples had rather similar In levels throughout storage. Post-rigor filleted SGS-MAP samples tended to have a slightly higher In level than the control, which may indicate that SGS had a positive effect on MA-packages as well. However, the amplified In values of post-rigor filleted SGS-MAP samples were not of a significant character. Both groups increased during the first samplings, followed by a decrease 7 days after filleting. By comparing the In levels of post-rigor filleted SGS-MAP and MAP samples 7 days after filleting with the Hx developments, it seems as if more In is converted into Hx in MAP samples. Even though post-rigor filleted SGS-MAP samples had a notably decrease in In 7 days after filleting, the amount of Hx seemed rather unaffected. SGS appears to slow down the microbial nucleotide degradation slightly, however, it is obvious that the advantage of SGS is most evident between vacuum and SGS-vacuum samples.

Hypoxanthine

Hx levels due to time of filleting

The raw-material of post-rigor filleted salmon had significantly higher levels of Hx compared to raw-material of pre-rigor filleted salmon. The results were expected as enzymes have had a longer period of time to be active in the post-rigor filleted salmon since measurements were taken 4 days post mortem. Although post-rigor filleted groups were initially higher, non of the

SGS processed groups were significantly higher. However, post-rigor SGS-MAP samples were slightly higher than the respective pre-rigor filleted group. Hence, the time of filleting also seems to have a minor impact on the microbial enzyme activity, except for conventional vacuum, which stood out negatively compared to the other groups. Consequently, SGS-treatment prior to vacuum packaging seems to have an important effect when filleting post-rigor. The SGS-treatment seems to improve the shelf-life considerably by inhibiting spoilage as the amount of Hx is significantly restrained.

Hx levels due to SGS-processing

Considering pre-rigor filleted samples in Figure 4.6a, there is a slight increase from raw-material until 7 days after filleting. Comparing this to APC and H_2S-producing bacteria, the counts are below the recommended quantification limit, with the latter not being present at all. Hence, little microbial enzyme activity is present. Huss *et al.* (1995) pointed out that even though microbial enzymes are mainly responsible for the formation of Hx, a slow accumulation of Hx can be caused by endogenous enzymes. As the SGS treated groups follow a similar path as the controls until 7 days after filleting, it emphasizes the previous statement of endogenous enzymes not being restrained by the presence of CO_2. Following the development further, both control groups have a notably increase in the amount of Hx 11 days after filleting, whereas SGS-vacuum and SGS-MAP samples follow the previous course. By taking APC into account, three of the pre-rigor filleted groups exceed the quantification limit 11 days after filleting; vacuum, MAP and SGS-MAP samples. Furthermore, H_2S-producing bacteria is for the first time detected in vacuum and SGS-MAP samples. The fact that SGS-MAP has equal APC counts as the control group, as well as H_2S-bacteria present, meanwhile the amount of Hx is unaffected, may imply that elevated amounts of CO_2, such as in the case of SGS, have an inhibiting effect on the microbial enzymes converting In into Hx. This also accords with the proposal on the inhibiting effect of CO_2 by Sivertsvik *et al.*(2002) on microbial enzymes.

Similar trends were observed for the post-rigor samples in Figure 4.6b. Already 3 days after filleting, Hx began to accumulate in the vacuum samples, whereas MAP, SGS-MAP, and SGS-vacuum only had a slight increase. Looking at the microbial growth, the APC given in Figure 4.9b had a markedly increase, exceeding the quantification limit in all groups at 7 days after filleting. Vacuum had the highest APC, which explains the high accumulation of Hx. The small increase from 3 to 7 days after filleting in SGS-MAP, MAP and SGS-vacuum samples, on the other hand, seemed to have little effect on the amount of Hx. As vacuum is the only group where CO_2 is completely absent, it seems reasonable that the presence of CO_2 in the three remaining groups is capable, to a certain extent, of preventing the microbial enzyme activity causing formation of Hx.

Interestingly, the Hx level of post-rigor filleted SGS-vacuum samples is not only significantly lower than vacuum samples 11 days after filleting, it is also significantly lower than MAP samples. The results imply that SGS-vacuum had a lower microbial enzyme activity compared to the samples stored in MAP. This further emphasizes the potential of replacing MA packages with vacuum packaging while maintaining the same or have an even better shelf-life when SGS treatment is applied. However, one should note that SGS-vacuum has a risk of bloating if pre-dissolved

CO_2 diffuse out of the tissue. This is problematic as the vacuum packaging may seem ruptured or the product may seem spoiled due to microbial gas production.

Following the development further, post-rigor filleted SGS-MAP and MAP had similar APC 11 days after filleting. The amount of Hx, however, is notably higher for MAP samples, though not significantly. As in the case of pre-rigor filleted groups, it seems that SGS-MAP samples are capable of maintaining a low amount of Hx, even though the APC is equal to those of vacuum and MAP samples. A plausible explanation is that the inhibiting effect of CO_2 is concentration-dependent. Unfortunately, the amount of dissolved CO_2 in the respective groups was not measured. This may have given a better indication and an explanation of why, for instance, SGS-vacuum samples have the lowest amount of Hx. One should also note that the APC from 7 to 11 days after filleting is decreasing, meanwhile the Hx amount keeps increasing, making APC a poor method of explanation. If the APC at 7 days after filleting is too high or APC at 11 days after filleting is too low, especially when considering SGS-MAP and MAP samples, which have Hx amounts situated between the vacuum and SGS-vacuum samples, is difficult to tell.

K-value

As K-value indicates the nucleotide portion of In and Hx, it reveals how far the autolytical degradation process has come. When comparing K-values of raw-material, it is reasonable that post-rigor filleted raw-material, which has been stored for 4 days as HOG before measuring, starts at a significantly higher level than pre-rigor filleted raw-material. However, as there are no common indices of K-values and the corresponding freshness state of the fish, it is more interesting to look at the development both within and between the groups rather than specific values.

K-value due to time of filleting

Even though no significant differences were present between pre- and post-rigor groups, the obtained K-values clearly outlined the rapid degradation in pre-rigor filleted samples compared to post-rigor filleted samples. Although the pre-rigor filleted start of significantly lower, the increase between 3 and 7 days after filleting is more rapid than in the post-rigor filleted samples. As previously mentioned, the initial autolytical changes are caused by endogenous enzymes, not microbial, and is accelerated by physical handling, such as early filleting. Furthermore, Surette *et al.* (1988) also confirmed that the breakdown rate of the nucleotide catabolites was greater in sterile fillets than in non-sterile gutted whole cod [36].

K-value due to SGS-processing

K-value quickly reached 100 %, hence few samplings illustrating the development of the groups. Thus, any pattern is difficult to determine as the arrangement of groups on specific sampling days were inconsistent. Furthermore, no significant differences were present between groups. However, despite the few data points, pre-rigor filleted SGS-vacuum had the overall highest K-value throughout the storage time, which is in line with the rapid decrease of IMP. However, as previously described, the amount of Hx of SGS-vacuum remained exceptionally low compared to the

other groups, consequently indicating that even though freshness is quickly lost, spoilage reactions are restrained. The low H-value of SGS-vacuum samples also confirms the latter suggestion. High K-value and its rapid increase is not unusual for some fish species. Huss (1995) reported that some fish species, for instance, Atlantic cod reach a maximum K-value well in advance of the shelf-life [12], thus emphasizing that K-value is a freshness and not spoilage indicator. Furthermore, when Luong *et al.* (1992) monitored K-values through storage, they revealed a rapid increase during the early stages, reaching 100% after 3 days of storage at 4°C.

H-value

The spoilage determining H-value is closely linked to the amount of Hx, which in turn is affected by the microbial enzyme activity. No proper H-value indices are available, and as the effectiveness of for instance MAP to increase shelf-life is dependent on the amount of solubilized CO_2 which in turn is dependent on the partial pressure of CO_2 and DF, a direct comparison of results is difficult when different systems are applied [61]. Furthermore, many studies do not state either DF or amount of solubilized CO_2, which makes it even more challenging to find comparable literature values. Moreover, when Fletcher *et al.* (2006) determined the spoilage of King Salmon, they concluded that more work needs to be done on the relationship between the hypoxanthine levels/H-values in salmon and sensory scores [118]. Nevertheless, SGS-processing seems to restrain the microbial enzymes as SGS-processed groups had lower H-values. The effect was most evident in the post-rigor filleted groups, especially between vacuum and SGS-vacuum samples. This finding is consistent with that of Sivertsvik (2000), who found SGS combined with vacuum packaging to have the longest shelf-life [93].

5.3 Microbiology

The initial APC and H_2S producing bacteria counts, obtained from this study, ranged from 0 log CFU/g to 1.36 log CFU/g. The low initial growth indicates that contamination during handling and processing was kept at a minimum. Furthermore, the low amounts of bacterial growth during the first samplings also indicates raw-material of good microbiological quality.

Aerobic plate counts

APC growth due to SGS-processing

When comparing the aerobic growth between pre-rigor filleted vacuum and SGS-vacuum samples, SGS-vacuum samples tended to have lower growth throughout storage as it was significantly lower on several samplings. Samples stored in SGS-MAP, on the other hand, had relatively similar APC as to those stored in MAP. Hence no effect of SGS seemed to be present. This outcome is contrary to that of Rotabakk *et al.* (2006, 2008), who found APC of MA-packed Atlantic Halibut and chicken breast fillets to be significantly reduced by SGS-processing [9, 10].

The gap between SGS-vacuum and vacuum samples, and the absent gap between SGS-MAP and MAP samples, might be caused by the presence of solubilized carbon dioxide. In the untreated vacuum samples, there is no carbon dioxide present at all, whereas for the untreated MAP samples, carbon dioxide is present. Consequently, conventional MA-packages have a certain defence against microbial growth, regardless of the SGS-treatment. Thus, it appears that the SGS treatment did not cause further resistance against the aerobically bacterial growth beyond the MA packaging. On the contrary, the growth in vacuum samples is on the same level as the fillets stored in MAP and SGS-MAP on several sampling days. This contradicts previous findings as the bacteriostatic effect is related to the amount of available CO_2 to dissolve into the product, i.e. the more dissolved CO_2 the higher the effect [9]. Following this statement, it would be reasonable if samples stored in vacuum, i.e. having no dissolved CO_2, had the highest growth. Meanwhile, the remaining groups should have been situated somewhere below, depending on the amount of dissolved product. Sampling day 26 is the only sampling which follows this reasoning.

When considering the post-rigor filleted groups, samples stored in SGS-vacuum were significantly lower than vacuum samples. Furthermore, the gap between vacuum samples and SGS-vacuum samples was even more prominent than in the pre-rigor samples. MAP and SGS-MAP samples were relatively equal during the entire storage; thus, as in the case for pre-rigor, SGS did not seem to have any major effect on MA-packages based on APC. It was also confirmed by the absence of correlation between CO_2 in headspace and APC. As post rigor SGS-vacuum samples also tended to have lower growth than both MAP and SGS-MAP, there is a potential of replacing MAP with SGS-vacuum, while still guarantying low microbial growth. By replacing MA-packages, where gas contributes to major parts of the volume, with vacuum packages, the product to packaging volume ratio increases tremendously.

APC growth due to time of filleting

Pre-rigor and post-rigor filleted samples had similar trends regarding the APC and no significant differences were found between groups of equal processing and packaging. However, the findings of the current study do not support the previous research of Rosnet *et al.* (2003) who reported significantly lower counts in pre-rigor filleted Atlantic salmon compared to post-rigor filleted.

Potential of postponing lag phase due to SGS-processing

At 3 days after filleting, post-rigor MAP and vacuum had higher amounts of APC growth than the SGS-treated groups. It may seem as if the SGS-treatment is capable of extending the lag phase, thus postponing the microbial growth. These results reflect those of Sivertsvik and Birkeland (2006), who found SGS-processing to prolong the lag phase of aerobic microorganisms [8]. Lengthening of the microbial growth lag phase due to the effect of CO_2 was also reviewed by Stammen *et al* (1990) [119]. Furthermore, it is also known that with increasing levels of CO_2, the rate of bacterial multiplication will decrease, in addition to increasing the length of lag phase [79]. However, following this statement, vacuum samples should have had the highest growth 3 days after filleting, not MAP, which has a certain amount of CO_2 present. It is important, however, to emphasize that the growth in MAP and vacuum linger between the recommended quantification limit, and the results may not be valid in terms of comparing the arrangement of

groups. Furthermore, several samplings during the supposedly lag phase are needed before any conclusions can be drawn.

H_2S producing bacteria

APC is not necessarily a good method to indicate spoilage as the microbial composition most likely also includes bacteria that do not cause spoilage. Thus, it is important to take the H_2S-producing bacteria into account, which are more often associated with spoilage. More prominent spoilage bacteria in fish, such as *Shewanella putrefaciens*, are H_2S-producing; thus the growth of these may give a better indication of how deteriorated the fish has become. Moreover, Fogart *et al.* (2019) suggested that the growth of H_2S-producing bacteria is a better indicator of spoilage than APC [120].

H_2S producing bacteria due to time of filleting

Pre-rigor and post-rigor filleted samples had distinct differences in the growth of H_2S-producing bacteria. The pre-rigor groups had clearly a higher presence of H_2S-producing bacteria than the post-rigor groups. These results are inconsistent with those of Rosnes *et al.* (2003), who reported pre-rigor filleted samples to have significantly lower counts of H_2S-producing bacteria compared to post-rigor filleted [68]. Overall, it is clear that there exists no simplistic explanation for the high H_2S-producing bacteria counts of pre-rigor filleted samples, versus the sporadically detected growth in the post-rigor filleted samples. Furthermore, Dalgaard *et al.* (1995) reported that the spoilage bacteria and H_2S-producing organism *S. putrefaciens* formed white colonies in IA. The findings indicate that not all H_2S-producing organisms form black colonies [60], and quantification of H_2S-producing bacteria is not always representative.

When comparing pre-rigor filleted groups against post-rigor filleted, post-rigor filleted SGS-vacuum samples definitely had the lowest growth as no H_2S-producing bacteria were detected. However, except for pre-rigor vacuum samples, most of the growth in both pre- and post-rigor groups were below the quantification limit in most of the storage time. No significant differences between pre- and post-rigor samples were present in the SGS-treated samples, hence indicating early filleting as an adequate approach when used in combination with SGS.

H_2S producing bacteria due to SGS-processing

In the pre-rigor filleted samples, SGS seemed to have the highest effect when combined with vacuum. Although pre-rigor SGS-vacuum was only significantly lower than pre-rigor vacuum at one sampling, 26 days after filleting, it tended to have a lower growth of H_2S-producing bacteria throughout storage. The growth in post-rigor samples were few and at too low amounts to make any conclusion. However, one could note that post-rigor filleted SGS-vacuum samples had no detectable growth, whereas post-rigor vacuum samples had growth at two samplings. Nevertheless, earlier findings by Rotabakk *et al.* (2008) found no effect of SGS-processing on H_2S-producing bacteria [9].

Adequacy of early filleting summary

An initial objective of the thesis was to identify the adequacy of early processing, i.e. pre-rigor filleting, compared to post-rigor filleting. No apparent trend was found between pre-rigor filleted and post-rigor filleted samples of equal processing and packaging method on APC, thus the growth seems unaffected by time of filleting time. On the contrary, the growth of H_2S-bacteria had more distinct differences. Although the H_2S growth was more prominent in pre-rigor samples, it was only significantly higher than the post-rigor filleted in the control groups. Consequently, pre-rigor filleting of SGS-processed salmon is most likely an adequate alternative to post-rigor filleting.

Furthermore, the growth of H_2S producing bacteria is low compared to the storage time and may not be of importance. Other degradation processes may lead to spoilage before H_2S-producing bacteria are capable of producing enough spoilage related compounds. Hence, the amount of H_2S-producing bacteria is not always the spoilage determining factor. This coincides well with the findings of Dewitt and Oliveira (2016) reviewing multiple papers where the spoilage determining factor was sensorial rejection, not microbial. [61] Additionally, Dalgaard *et al* (1993) discovered that spoiled vacuum packed and MAP cod fillets had TMA and ammonia-like off-odours. In contrast to aerobically stored cod, the sensory characteristics revealed no sulphidy off-odours. This was supported by chemical analysis revealing large amounts of TMA, meanwhile the H_2S and CH_2SH amount in the spoiled packed fish were very little [52]. Hence, H_2S-producing bacteria is not always a proper indicator of shelf-life. Additionally, Dalgaard (1995) reported that some strains of H_2S producing bacteria appeared as white colonies on IA [60], hence the black counts may not be entirely representative for the H_2S-producing bacteria counts.

5.4 Colour

Color characteristics evaluated according to time of filleting

There were no significant differences in lightness (L*), the intensity of redness (a*), or yellowness (b*) between raw-material of pre-rigor filleted salmon and post-rigor filleted salmon, though the pre-rigor filleted were in general slightly lighter. Nevertheless, the main focus in the following section will be comparing the pre- and post-rigor SGS-processed groups.

All pre-rigor and post-rigor SGS-processed groups were significantly lighter (L*) and had a significant increase in redness (a*) and yellowness (b*) compared to the raw-material ($p<0.026$). Hence, an alteration in color of SGS-processed groups seems inevitable. Taking storage time into account, time of filleting did not cause any significant differences in neither lightness (L*), redness (a*), nor yellowness (b*) between the SGS-processed pre-rigor and post-rigor filleted groups. Although no significant differences were present, it should be noted that throughout storage, pre-rigor SGS vacuum samples had a 1.6 point higher average score than post-rigor SGS vacuum samples. This contradicts previous findings of Skjervold et al. (2001) were lightness (L*) of pre-rigor filleted salmon was significantly lower than the post-rigor filleted, throughout the storage time [121]. However, the fillets in the report were stored under other conditions and were not SGS-processed, additionally, a different apparatus, Minolta Chroma Meter II-CR200, were used to record the CIE values[112]. Thus, the results may not be directly comparable.

In brief, pre-rigor filleted SGS-MAP and SGS-vacuum had insignificant differences to post-rigor SGS-MAP and SGS-vacuum, respectively. In other words, the SGS-treatment seems to be an adequate method regardless of the time of filleting, in terms of color characteristics.

Color characteristics evaluated according to SGS-processing

Except for the significantly higher redness (a*) of pre-rigor filleted SGS-MAP samples compared to pre-rigor MAP samples, the SGS-treatment did not have any significant effect on lightness (L*), redness (a*), and yellowness (b*) of neither MA-packed nor vacuum packed samples. This coincides well with the findings by Rotabakk *et al.* (2006, 2008) stating that fillet color was not affected by SGS-processing [9, 10]. Yet, SGS-processed products seemed to be generally lighter (L*) than the conventional packed samples. As CO_2 has been found to lighten products during storage due to its acidic properties [108], it was hypothesized that lightening of a product could be dependent on the CO_2 concentration. Accordingly, SGS-processed groups were assumed to be lighter than the controls and, together with MAP, to be lighter than vacuum. However, the concentration of CO_2 did not seem to be decisive for the lightness appearance.

An overall trend was the significant differences between raw-material and the remaining samplings, irrespectively of the packaging method and SGS-treatment. Even though the initial conditions may be deemed as the most wanted, color changes will occur post-mortem due to the altered physical state of the myofibrils, according to Erikson and Misimi (2008). These changes will affect the light scattering properties as muscle/cell structure is affected by the pH decrease and rigor contractions. Thus, these color changes are inevitable and, therefore, to be expected.

In short, only minor insignificant differences were found between SGS-treated samples and their control groups. In general, as neither lightness (L*), redness (a*), or yellowness (b*) were negatively affected by the elevated CO_2 concentrations, there is no disadvantageous color change caused by the SGS-processing.

5.5 Demand and future potential

Consumer Demand

Considering consumer demand, one should ask if there is any interest in buying SGS products. Even if the product is not spoiled after 30 days, would consumers still find it appealing? Will the appearance, i.e. color and texture, still be appetizing, and can a 30 days old fish still be classified as fresh? These are important issues to investigate in future research.

Industrial Demand

As Norwegian salmon is often considered of superior quality, one should question if there is any demand for SGS-technology. If the technology is practically feasible at low cost in industrial scale processes, shelf-life extension and potentially a lower DF is exclusively a positive effect and most likely a wanted feature. Further testing should be conducted to reveal, not only shelf-life

extension effects, but also the cost and feasibility of the method. Moreover, there is a potential of implementing the technology to other industries, for instance, to meat-products, and further research is suggested.

Future potential

Further research should be undertaken to investigate the potential of increasing DF by either reducing the amount of headspace gas of SGS-packed products, consequently reducing the package size, or substitute conventional MAP with SGS-vacuum packaging.

There is, at present, no proper understanding of how the growth of specific species of spoilage bacteria are affected by the SGS-treatment. In order to determine the shelf-life of the product, knowledge of the present microorganisms and the magnitude is crucial. If SSO are not present among the microbiota, then the product is not necessarily spoiled even though the APC might indicate so due to high CFU/g values. The H_2S-producing bacteria counts give a certain intuition of the shelf-life as these bacteria produce undesirable compounds. However, varying spoilage counts of H_2S producing bacteria have been reported as it depends on the species. A further study with more focus on the composition of the microbiota is therefore suggested.

Chapter 6

Conclusion

This study set out to investigate the effects of SGS processing during storage and if the effect was affected by time of filleting. To answer the first objective, this study has shown that there exists a potential of combining SGS technology with conventional vacuum packaging in order to restrain the microbial growth and postpone spoilage reactions.

The most prominent finding to emerge from this study is that compounds associated with the activity of microbial enzymes, such as inosine and hypoxanthine, as well as H-values, were reduced in SGS-vacuum samples compared to the control. The microbial enzyme activity appears to be restrained by the SGS-processing. Furthermore, the APC and H_2S producing bacteria counts of SGS-processed vacuum samples were also reduced. Thus SGS combined with vacuum packaging seemed to restrain the microbial growth as well. On the contrary, the elevated CO_2 concentrations in SGS-MAP packaging did not affect the APC. Hence SGS combined with MAP packaging had no inhibiting effect on the aerobically microbial growth.

The research has also shown that SGS-processing made no significant difference to the endogenous enzyme activity as the amount of IMP and K-values were unaffected by SGS-processing. As freshness is mainly associated with the action of endogenous enzymes, and spoilage is commonly known to be caused by microbial enzymes, it is concluded that SGS-processing salmon fillets prior to packaging affect the overall storage quality in vacuum packed products in terms of spoilage, not freshness.

SGS-processing appears to have a certain effect on storage quality of MAP packaging. However, it is obvious that the advantage of SGS is most evident when combined with vacuum packaging. Although SGS-processed MAP samples had a significantly higher amount of CO_2 in headspace compared to the control group, the significance of the elevated headspace CO_2 concentration on APC, H_2S-producing bacteria and pH were marginal. However, the SGS-treatment may have affected the microbial enzymes accountable for the conversion of IMP into In and Hx. The post-rigor filleted SGS-vacuum samples, on the other hand, had no detectable H_2S-producing bacteria. Although the growth of H_2S-producing bacteria was notably higher in all the pre-rigor groups, pre-rigor SGS-vacuum samples were, in general, able to remain below the recommended quantification limit. The distinct differences between SGS-treated and untreated vacuum samples confirm

the bacteriostatic effect of CO_2 and how the novel vacuum packaging can be utilized to achieve enhanced storage quality.

An interesting finding to emerge from this study is the potential of utilizing SGS-vacuum as a substitute for MA-packages, while at the same time guarantee low microbial growth and limit the spoilage. The significantly lower APC, H_2S-producing bacteria count, amount of Hx and H-value in SGS-vacuum samples compared to MAP-samples emphasize the adequacy of replacing MAP with SGS-vacuum packaging. Several beneficial aspects are associated with using the novel approach of SGS-vacuum packaging above conventional MAP. For instance, a higher DF can be obtained and a reduced amount of required packaging. These are desirable features from an economical, as well as environmental, point of view. Consequently, as MAP is frequently used, SGS-technology combined with vacuum could potentially become a highly sought after technique, if acknowledged by industry.

In general, the SGS-processing of MAP and vacuum packed salmon did not have any significant effect on the fillet lightness(L*), redness (a*), and yellowness (b*). Pre-rigor SGS-MAP samples were the only exception by having a significantly higher fillet redness (a*) than the control. However, the significance of these results on quality is assumed marginal.

The second aim of the present research was to examine the adequacy of combining the SGS-treatment with early filleted salmon, i.e. pre-rigor filleting. No significant differences were present between post-rigor and pre-rigor in the case of CO_2 amount in headspace, pH and APC. On the contrary, the H_2S producing bacteria were sporadically detected in the post-rigor filleted groups, meanwhile pre-rigor filleted groups had notable growth, though mainly below the quantification limit. Existing theories are not able to explain why pre-rigor and post-rigor filleted samples are so dissimilar in terms of H_2S producing bacteria counts, thus it still remains unclear.

As endogenous enzymes are present and active during the resolution of rigor, pre-rigor filleting may be a better option. However, the IMP level was decreasing rapidly, resulting in a rapid increase of K-value. As pre-rigor filleting accelerate the autolytical process, the freshness quickly diminished. In terms of spoilage, no significant differences in Hx and H-value of SGS-processed groups were present. However, if post-rigor filleting is to be applied, SGS-treatment seems to be of special importance in vacuum packed samples as the levels of Hx and the H-values of conventional vacuum were significantly higher than SGS-vacuum samples.

As all variations in the color parameters between pre-rigor SGS-MAP and SGS-vacuum and their respective post-rigor groups were insignificant, time of filleting seemed not to affect the color appearance of SGS treated salmon. Thus, pre-rigor filleting can be utilized in preference to post-rigor filleting, without causing color changes of the SGS-treated salmon.

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Appendix

6.1 Raw material

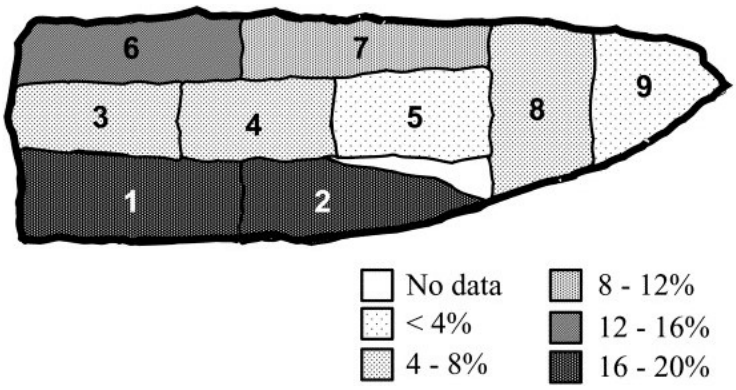


Figure A.1: Schematic illustration of the lipid distribution in salmon. Areas with approximately equal lipid content are grouped. The figure is obtained from [122].

6.2 Gas composition in MAP

Table A.1: The gas compositions of the SGS system, the five MAP-dummies, and the five SGS-MAP dummies were analyzed to ensure the correct atmosphere inside the package. The measurements are given as mean ± S.D., with the exception of SGS systems as $n = 2$.

Pre-rigor	CO_2 [%]	O_2 [%]	N_2 [%]
SGS-MAP	60.3 ± 0.4	0.04 ± 0.02	39.6 ± 0.4
MAP	60.70 ± 0.13	0.02 ± 0.01	39.26 ± 0.13
SGS system	95.4 - 95.7	0.49 - 0.57	3.8 -4.0
Post-rigor			
SGS-MAP	59.8 ± 0.3	0.068 ± 0.01	40.2 ± 0.3
MAP	60.2 ± 0.2	0.02 ± 0.01	39.8 ± 0.21
SGS system	94.8 - 96.3	0.42 - 0.78	3.3 - 4.4

6.3 Air pressure during packaging and storing

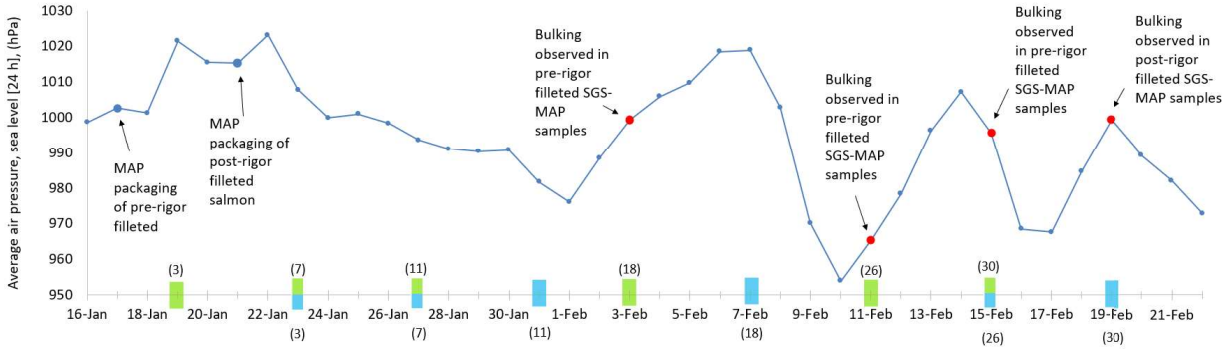


Figure A.2: The average air pressure of 24 h at sea level from 16.01.2020 (date of slaughter) until 22.02.2020 (Last sampling of post-rigor filleted samples 30 days after filleting). The green marks imply samplings of pre-rigor filleted salmon, and blue marks imply samplings of post-rigor filleted salmon. Numbers in parenthesis indicates days after filleting. Data were collected from the Norwegian Centre for Climate Services (NCCS) [123].

6.4 Analysis of ATP degradation compounds

The area, obtained from the integration of the peak, was plotted for each metabolite against its associated concentration. ATP and IMP had concentrations below 1000 μmol/L as they both were in a state of salt and needed to be recalculated. A linear regression with an intersection through origin was applied to the data set. The outcome is given below in Figure A.3.

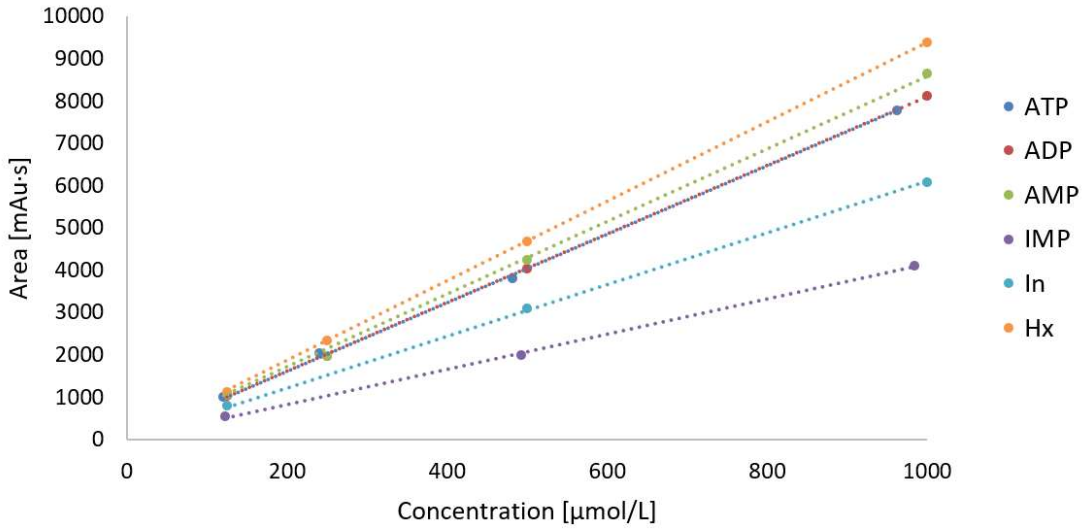


Figure A.3: Graphical illustration of the peak areas plotted against known concentrations of given metabolites. Orange = hypoxanthine, green = AMP, red = ADP, blue = ATP, cyan = inosine, purple = IMP. The dotted lines represent the linear regression through the origin.

Table A.2: Tabled values of retention time, R_t, detection level, coefficient of determination,R^2, and linear equation to ATP and its derivatives. The interception of the linear regression was set through the origin.

Metabolite	Detection [nm]	$R_{t.1}$ [min]	$R_{t.2}$ [min]	$R_{t.3}$ [min]	y = ax +b	R^2
ATP	210	7,34	7,92	8,6	y = 8,07 x	0,9994
ADP	210	5,83	6,03	6,21	y = 8,10 x	0,9999
AMP	260	4,44	4,48	4,54	y = 8,58 x	0,9989
IMP	260	3,16	3,24	3,25	y = 4,14 x	0,9994
In	260	3,79	3,66	3,66	y = 6,11 x	0,9997
Hx	260	2,80	2,79	2,76	y = 9,37 x	0,9999

6.5 ATP degradation compounds due to time of slaughter

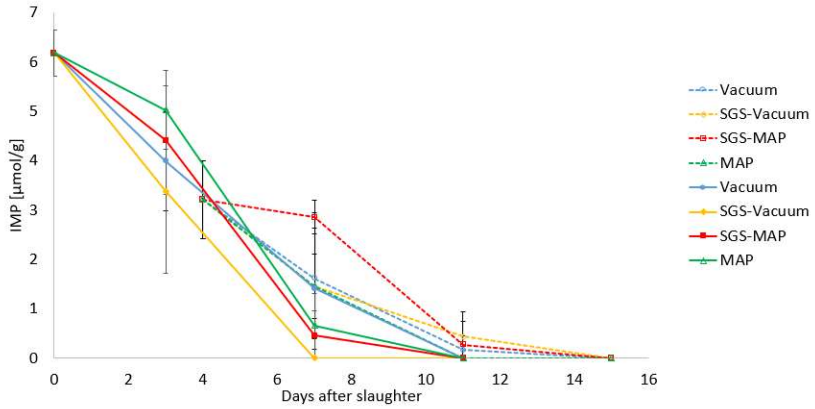


Figure A.4: Concentrations of IMP in samples filleted pre-rigor: vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■); and samples filleted post-rigor: vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The data is presented as mean values and error bars represent S.D. (*n*=3).

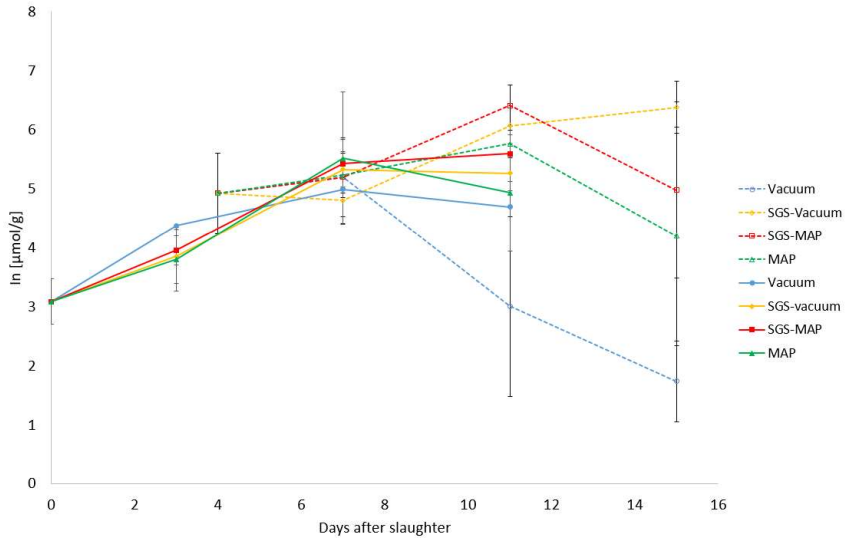


Figure A.5: Concentration of inosine (In) in pre- and post-rigor salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The error bars represent S.D. (*n*=3)

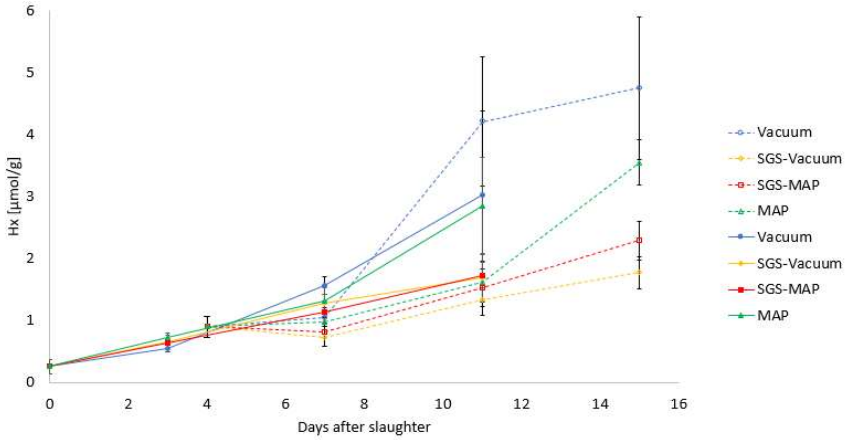


Figure A.6: Concentration of hypoxanthine (Hx) in pre- and post-rigor salmon throughout storage. Pre-rigor filleted samples are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲) and SGS-MAP (■). Post-rigor filleted samples are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The error bars represent S.D. (*n*=3)

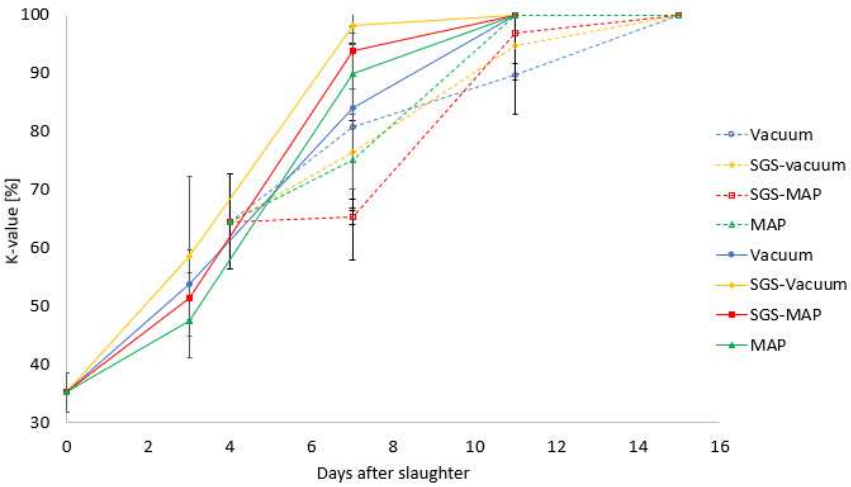


Figure A.7: K-values of pre-rigor and post-rigor filleted salmon samples throughout the storage time. The pre-rigor filleted groups are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲), SGS-MAP (■), the post-rigor filleted groups are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The K-values were calculated using equation 2.1. The K-values are presented as average values and the error bars represent S.D. (n=3)

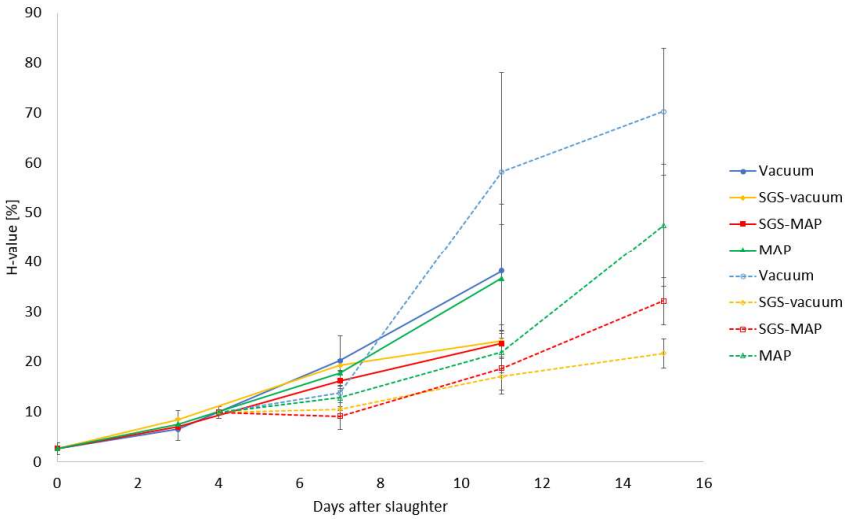


Figure A.8: H-values of pre-rigor and post-rigor filleted salmon samples throughout the storage time. The pre-rigor filleted groups are indicated as vacuum (●), SGS-vacuum (◆), MAP (▲), SGS-MAP (■), the post-rigor filleted groups are indicated as vacuum (○), SGS-vacuum (◇), MAP (△), SGS-MAP (□). The H-values were calculated using equation 2.2. The H-values are presented as average values and the error bars represent S.D. (n=3)

6.6 Color

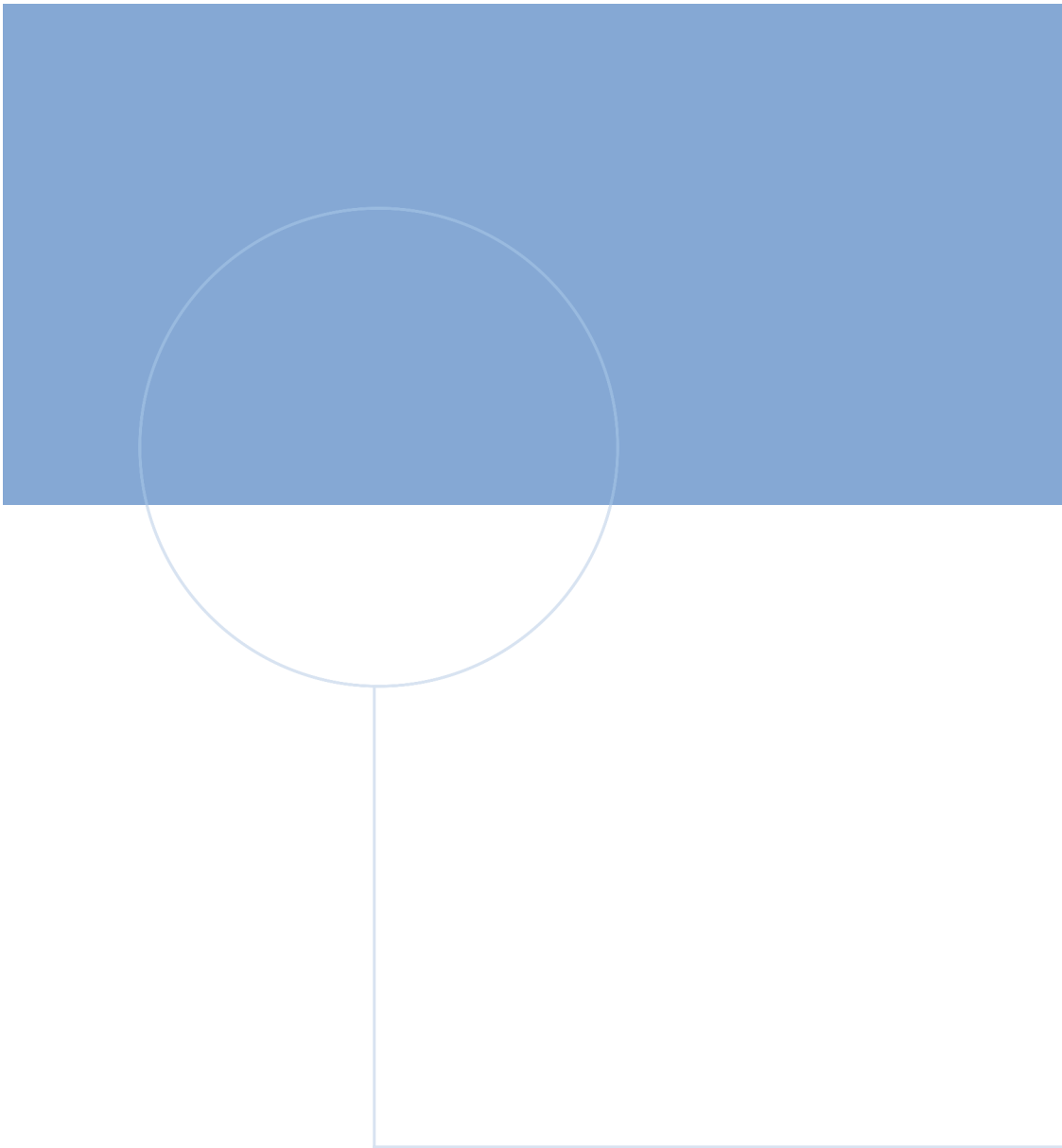
Table A.3: The effect of SGS-processing on surface color of pre-rigor filleted salmon stored in MAP and vacuum packaging. Results are shown as means ± SD. Presented values of day 11 and 30 are mean of 3 individual samples per group. Day 0 presents mean values of raw-material from 5 individual pre-rigor filleted salmon. L* describes lightness, a* is redness and b* is yellowness. Significant differences ($p<0.05$) between groups[1] (a, b) and days[2] (x, y)were tested by one-way ANOVA.

Parameter	day	vacuum (control)	SGS-vacuum	MAP (control)	SGS-MAP	p-value[1]
L*	0	57.9 ± 0.8^{x}	57.9 ± 0.8^{x}	57.9 ± 0.8^{x}	57.9 ± 0.8^{x}	-
	11	64.6 ± 1.7^{y}	64.7 ± 0.5^{y}	59.4 ± 0.8^{x}	63.0 ± 3.6^{y}	0.044*
	30	63 ± 2^{y}	65.6 ± 1.1^{y}	62.2 ± 1.3^{y}	64.4 ± 1.0^{y}	0.072
	p-value[2]	0.000	0.000	0.001	0.004	
a*	0	13.1 ± 1.5^{x}	13.1 ± 1.5^{x}	13.1 ± 1.5^{x}	13.1 ± 1.5^{x}	-
	11	$18.6 \pm 0.7^{ab,y}$	$19.5 \pm 0.5^{b,y}$	$14.6 \pm 1.1^{a,x}$	$19 \pm 3^{ab,y}$	0.024
	30	$17.4 \pm 1.1^{a,y}$	$20.34 \pm 0.03^{b,y}$	$18.1 \pm 1.1^{a,y}$	$19.5 \pm 0.7^{ab,y}$	0.011
	p-value[2]	0.001	0.000	0.003	0.002	
b*	0	11 ± 2^{x}	11 ± 2^{x}	11 ± 2^{x}	11 ± 2^{x}	-
	11	$15.6 \pm 0.6^{ab,y}$	$15.8 \pm 0.2^{b,y}$	$13 \pm 2^{a,xy}$	$15.3 \pm 1.0^{ab,y}$	0.038
	30	15.3 ± 0.4^{y}	15.2 ± 0.3^{y}	15.9 ± 0.4^{y}	16.0 ± 0.5^{y}	0.071
	p-value[2]	0.009	0.006	0.026	0.007	

*one-way ANOVA gave significant differences between groups, however, the post hoc Tukeys' multiple comparison test was not able to distinguish them. When post-hoc Duncan test was applied, the following arrangements were obtained; MAP samples (a), SGS-MAP samples (ab), vacuum (b), and SGS-vacuum (b).

Table A.4: The effect of SGS-processing on surface color of post-rigor filleted salmon stored in MAP and vacuum packaging. Results are shown as means ± SD. Presented values of day 11 and 30 are mean of 3 individual samples per group. Day 0 presents mean values of raw-material from 5 individual post-rigor filleted salmon. L* describes lightness, a* is redness and b* is yellowness. Significant differences ($p<0.05$) between groups[1] (a, b) and days[2] (x, y) were tested by one-way ANOVA.

Parameter	day	vacuum (control)	SGS-vacuum	MAP (control)	SGS-MAP	p-value[1]
L*	0	57.3 ± 1.3^{x}	57.3 ± 1.3^{x}	57.3 ± 1.3^{x}	57.3 ± 1.3^{x}	-
	11	60.5 ± 2.1^{y}	63.0 ± 0.9^{y}	63.7 ± 2.1^{y}	65.0 ± 3.6^{y}	0.217
	30	63.1 ± 1.3^{y}	64.0 ± 1.2^{y}	66.1 ± 1.5^{y}	63.7 ± 2.5^{y}	0.226
	p-value[2]	0.001	0.000	0.000	0.002	
a*	0	11.4 ± 1.3^{x}	11.4 ± 1.3^{x}	11.4 ± 1.3^{x}	11.4 ± 1.3^{x}	-
	11	16.2 ± 2.7^{y}	18.5 ± 1.1^{y}	18.0 ± 2.7^{y}	19.2 ± 2.3^{y}	0.338
	30	16.4 ± 1.3^{y}	17.9 ± 0.9^{y}	19.1 ± 0.7^{y}	17.9 ± 1.5^{y}	0.106
	p-value[2]	0.011	0.000	0.000	0.001	
b*	0	9.6 ± 0.2^{x}	9.6 ± 0.2^{x}	9.6 ± 0.2^{x}	9.6 ± 0.2^{x}	-
	11	14.6 ± 1.7^{y}	15.7 ± 0.1^{y}	15.3 ± 1.7^{y}	14.6 ± 0.9^{y}	0.468
	30	14.8 ± 0.2^{y}	15.2 ± 0.5^{y}	13.9 ± 0.9^{y}	14.9 ± 0.5^{y}	0.101
	p-value[2]	0.011	0.003	0.008	0.008	



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