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FEUP FACULDADE DE ENGENHARIA
UNIVERSIDADE DO PORTO

SOLUBLE GAS STABILIZATION ON COOLED HEAT-PROCESSED FISH PRODUCTS, EFFICACY AND SHELF-LIFE EFFECT

MOISÉS CARDOSO COUTO
MASTER THESIS IN CHEMICAL ENGINEERING
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Master in Chemical Engineering

Soluble gas stabilization on cooled heat-processed fish products, efficacy and shelf-life effect

Master dissertation

of

Moisés Cardoso Couto

Developed within the course of dissertation

held in

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NTNU

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Abstract

Food waste is a significant contributor to global food insecurity with economic and environmental impacts. One way to address this issue is by extending the shelf-life. However, shelf-life extension tendentially means an increase in processing, mainly for perishable foods, such as fish, which is not desirable by consumers.

Due to this reason, the need for novel technologies to extend shelf-life while impacting the product's quality is urgent. Carbon dioxide (CO_2) dissolution on food by modified atmosphere packaging is a great option. Nonetheless, it has a physical constraint due to CO_2 solubility, which can damage packages and products if surpassed.

In order to overcome that constraint, a CO_2 dissolution process known as soluble gas stabilization (SGS) on a fish product (fish cakes) was studied. The focus was on its interaction with thermal processes and comparison of influence of temperature in its efficiency and efficacy. Fish cakes were treated hot, just after heat treatment, and cold, after cooling in three time frames (0.5, 1.0 and 1.5 h) to compare the technology time efficiency and efficacy in extending the shelf-life and preserving quality.

The efficiency, studied on the gas solubility through a gravimetric method, showed better results with the treatment on a hot product, which achieved 1076.5 ± 83.3 ppm, or 36 % of the maximum solubility in the first half hour, while the treatment on cold only achieved 5 %. Moreover, a little higher solubility rate was found in the following half hours on the still cooling samples (>6 %) than in the ones already in the equilibrium temperature (<5 %).

The effect on shelf-life, indicated by the microbial stability of the product, was very pronounced. An extension for 10 days (from the original 30) was obtained from a 90 minute hot treatment and doubled to 60 days in with an overnight treatment. The quality was preserved as the treatment had close to no impact on the pH. Additionally, the drip loss was slightly reduced and the texture was subtly enhanced.

This work made visible the SGS technology as a promising microbial hurdle and very synergetic with thermal processes to answer the challenges of preserving food, mainly in fish products.

Keywords (theme): Carbon dioxide; Soluble gas stabilization; microbial shelf life; fish cakes; thermal processing; seafood.

Resumo

O desperdício de alimentos é um contribuinte significativo para a insegurança alimentar global, com impactos econômicos e ambientais. Uma maneira de abordar essa questão é estendendo a vida útil dos alimentos, no entanto, a extensão do tempo de prateleira geralmente implica em um aumento no processamento, principalmente para alimentos perecíveis, como peixes, o que não é desejável pelos consumidores.

Diante disso, a necessidade de novas tecnologias para estender o tempo de prateleira dos alimentos, ao mesmo tempo em que impacta a qualidade do produto, é urgente. A dissolução de dióxido de carbono nos alimentos por meio de embalagens com atmosfera modificada é uma ótima opção, no entanto, possui uma fronteira física devido à solubilidade do CO_2, que pode danificar embalagens e produtos se ultrapassada.

Para contornar essa fronteira, um processo de dissolução de CO_2 conhecido como estabilização de gás solúvel (SGS) em um produto de peixe (bolos de peixe) foi estudado. O foco foi em sua interação com processos térmicos, comparando a eficiência e eficácia do tratamento com a temperatura como principal fator. Os bolos de peixe foram tratados a quente, logo após o tratamento térmico, e a frio, após o resfriamento em três durações (0,5, 1,0 e 1,5 horas) para comparar a eficiência em tempo e a eficácia da tecnologia na extensão do tempo de prateleira e preservação da qualidade.

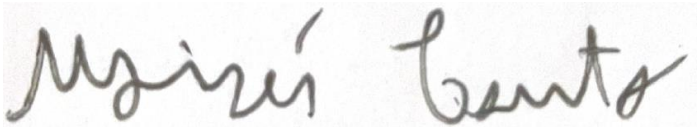
A eficiência, estudada pela solubilidade do gás por meio de um método gravimétrico, mostrou melhores resultados com o tratamento a quente. Este, alcançou 1076,5 ± 83,3 ppm, ou 36 % da solubilidade máxima na primeira meia hora, enquanto o tratamento a frio alcançou apenas 5 %. Além disso, foi observada uma taxa um pouco maior de solubilidade nas meias horas seguintes nas amostras ainda em resfriamento (>6 %) do que nas que já estavam em temperatura de equilíbrio (<5 %).

O efeito na vida útil, indicado pela estabilidade microbiana do produto, foi muito pronunciado. Uma extensão de 10 dias (dos originais 30) foi obtida a partir de um tratamento a quente de 90 minutos e duplicada para 60 dias com um tratamento durante a noite. A qualidade foi preservada, uma vez que o tratamento teve um impacto próximo de zero no pH, a perda de gotejamento foi ligeiramente reduzida e a textura foi sutilmente aprimorada.

Este trabalho tornou visível a tecnologia SGS como um promissor obstáculo microbiológico e muito sinérgico com os processos térmicos, visando responder aos desafios da preservação de alimentos, principalmente em produtos de peixe.

Declaration

I hereby declare, under word of honour, that this work is original and that all non-original contributions is indicated and due reference is given to the author and source



Moisés Cardoso Couto, July 1st 2023

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Notation and Glossary

$C_{CO_2}^{t=\infty}$	total CO_2 (ppm) absorbed by the product	ppm
$H_{CO_{2,P}}$	temperature-dependent Henry's constant for CO_2 in the sample	Pa×ppm^{-1}
m_{max}	maximum specific growth rate	
$MmCO_2$	molecular mass of CO_2	
P	absolute pressure	Pa
$P_{CO_2}^{t=\infty}$	partial equilibrium pressure of CO_2 in the headspace gas	Pa
R_a	Rate of absorption	s^{-1}
T	Temperature	K
$V_g^{t=0}$	gas volume at start	m^3
$V_g^{t=\infty}$	gas volume at equilibrium	m^3
R_a	example of a dimensionless number	
W_f	weight of the product	kg

List of Acronyms

APC	Aerobic Plate Count
CFU	Colony Forming Units
DL	Dip Loss
FAO	Food and Agriculture Organization
FD	Filling Degree
HTST	High Temperature Short Time
MA	Modified Atmosphere
MVH	Microwave Volumetric Heating
SGS	Soluble Gas Stabilization
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
UHT	Ultra-High Temperature
WHC	Water-Holding Capacity

1 Introduction

1.1 Framing and presentation of the work

1.1.1 Food chain scenario: food spoilage

Food security is a growing global concern due to the increasing population, climate change, and changing dietary habits. The United Nations defines food security as "when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life." Unfortunately, the current state of food security worldwide does not align with this definition.

According to the Food and Agriculture Organization (FAO) of the United Nations, an estimated 811 million people suffered from chronic undernourishment between 2019 and 2021. This number has been rising since 2014, as shown in Figure 1.1, with the COVID-19 pandemic exacerbating the problem due to disruptions in food supply chains and economic downturns. Moreover, food insecurity disproportionately affects vulnerable populations, including women, children, and people living in poverty (FAO, 2021).

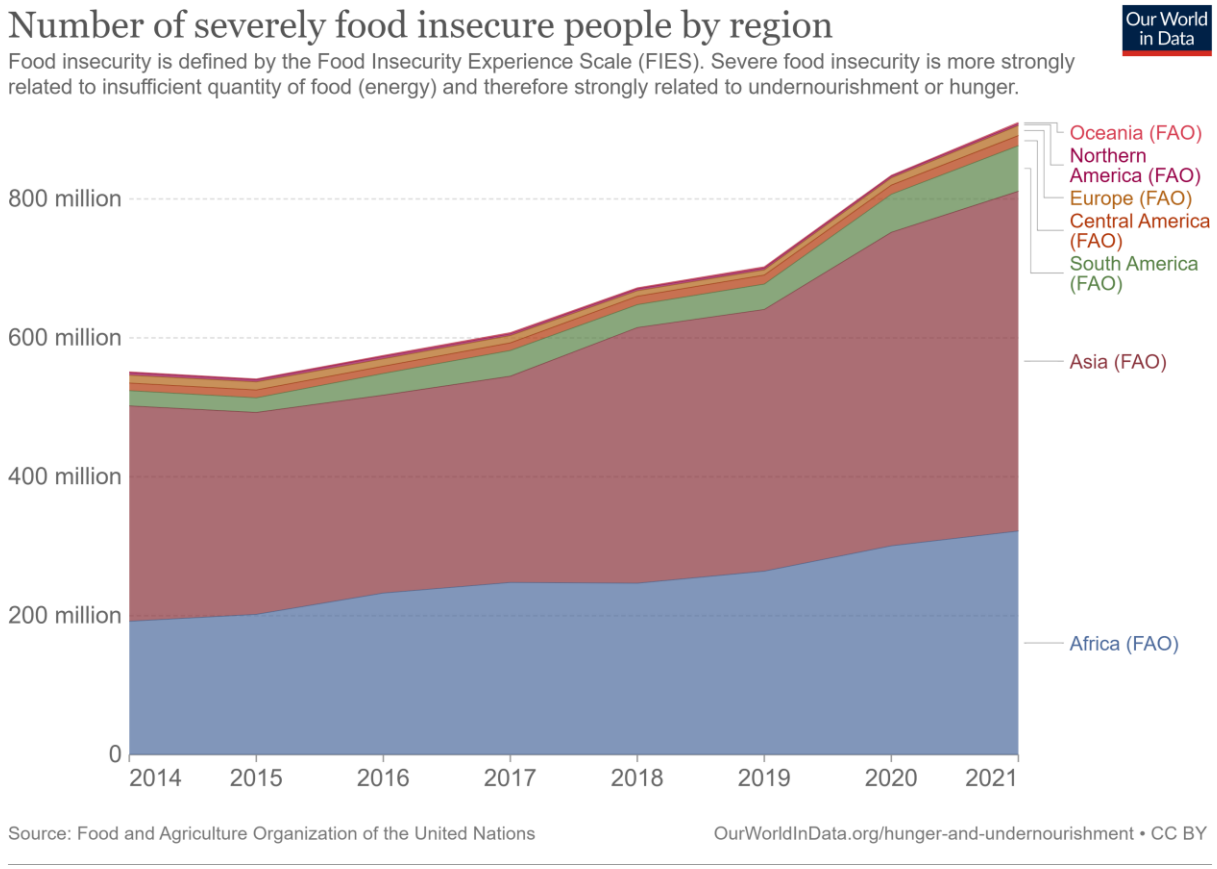

Figure 1.1 Global food insecurity along time. Source: FAO (2021). Adapted from https://ourworldindata.org/grapher/number-of-severely-food-insecure-people-by-region .

Food waste is a significant contributor to global food insecurity, with up to one-third of all food produced being wasted each year (Gustavsson et al., 2011). This represents a tremendous waste of resources, including water, land, energy, and labor, that went into producing, processing, and distributing food. The environmental impact of food waste is far-reaching. The decomposition of food waste in landfills produces methane, a potent greenhouse gas that contributes to climate change. In fact, food waste is responsible for approximately 6 % of global greenhouse gas emissions, with detailed participation in Figure 1. 2 (Poore & Nemecek, 2018). Moreover, the resources used in food production, such as water and fertilizers, are wasted when food is discarded. This not only puts unnecessary pressure on natural resources but also exacerbates issues of water scarcity and pollution. One way to address this issue is by extending the shelf life, length of time that a food product stored, displayed, or consumed without significant degradation or spoilage, of perishable goods to reduce spoilage and waste.

Figure 1.2 Distribution of greenhouse gas emissions in food production. Source: Poore and Nemecek (2018). Adapted from https://ourworldindata.org/food-waste-emissions .

On the other side of the problem, through the eye of the food industry, preserving the freshness and quality of perishable goods is essential for ensuring that consumers have access to safe and high-quality products. However, maintaining the quality of perishable goods can be challenging due to the rapid growth of microorganisms and the oxidation of the product. This can lead to spoilage, which not only reduces the nutritional value of the product but also poses a potential health hazard. One solution to this problem is the use of packaging to create a controlled atmosphere that slows down the growth of microorganisms and the rate of oxidation, thereby extending the shelf life of the product (Singh & Anderson, 2004).

Food spoilage and waste is also a problem faced by consumers, many find it frustrating to throw away food that has expired or gone bad, as it not only wastes their money but also contributes to the environmental issues presented above. An extended shelf-life, allows them to reduce

food waste and make more efficient use of their purchases. This benefits consumers by providing them with greater flexibility in meal planning and reducing the frequency of restocking trips to the grocery store, enhancing the convenience factor for consumers. With longer shelf life, consumers can stock up on perishable goods without the fear of them spoiling quickly. This is particularly beneficial to the contemporary culture of productivity or those living in remote areas with limited access to fresh products. It allows consumers to have a wider variety of perishable goods readily available, promoting convenience and reducing the need for frequent grocery shopping trips (Amani & Gadde, 2015).

1.1.2 Norway and the aquatic food

Norway, with its thriving seafood industry, offers a unique perspective on these challenges and opportunities. Renowned for its rich marine resources and sustainable seafood production, it plays a vital role in the global seafood market, being the second largest exporter and this industry being the second biggest in the country (Straume et al., 2020).

The Norwegian aquatic food industry faces specific challenges in maintaining the quality and freshness of the products throughout the supply chain while meeting the increasing global demand for sustainable seafood and ensuring the long-term viability of its marine resources. Fish products are highly perishable, unlike some other food products, such as grains or canned goods, they are more susceptible to spoilage due to their composition and inherent characteristics. One of the primary factors contributing to the perishability of fish is its high-water content. The moisture content creates an ideal environment for the growth of bacteria and other microorganisms, accelerating spoilage. This makes careful handling, proper storage, and efficient transportation essential to preserve the quality and prevent the spoilage of seafood products (Bergesen & Tveterås, 2019).

In addition to high moisture content, seafood contains natural enzymes that can lead to enzymatic degradation. For instance, fish contains proteases and lipases, which can break down proteins and fats, resulting in changes in texture, flavor, and appearance. These enzymatic reactions can lead to the development of off-flavors, rancidity, and undesirable textural changes in seafood products. Furthermore, aquatic food is highly susceptible to temperature abuse. Inadequate temperature control during transportation and storage can promote bacterial growth and enzymatic activity, accelerating the deterioration and spoilage of seafood. Temperature management is crucial to minimize the growth of spoilage-causing microorganisms and maintain the quality and freshness of seafood products. In order to ensure the quality and freshness of seafood products, the industry implements stringent protocols and quality control measures at every stage of the supply chain, prioritizing temperature control and minimizing the time between harvest and delivery (Alasalvar et al., 2011).

By addressing the challenges associated with the perishability of seafood, Norway can not only enhance the profitability and competitiveness of its seafood industry but also contribute to global food security and sustainability. By reducing the amount of seafood wasted due to spoilage, Norway can meet the increasing global demand for nutritious seafood while minimizing the environmental impact of the industry. Efforts to extend the shelf life and maintain the quality of seafood products play a crucial role in ensuring the availability of safe and high-quality seafood for consumers worldwide.

1.1.3 Modified Atmosphere Packaging and Soluble Gas Stabilization

As seen, the extension of the shelf life is a vital piece for many puzzles of the food chain. In addressing the challenges faced by the Norwegian seafood industry and the global food industry as a whole, one promising solution lies in the utilization of packaging, which is also increasingly important due to the global demand for fresh and high-quality products. Packaging can also reduce food waste by extending the shelf life of products, which is essential for countries that experience logistical challenges in getting products to consumers quickly. Additionally, longer shelf life reduces the need for frequent restocking, which can improve efficiency and reduce costs for retailers. Particularly, modified atmosphere (MA) packaging technology aims to create an optimal gas composition within the packaging environment, thereby extending the shelf life of perishable goods and preserving their quality (Abel, 2021).

MA packaging is a preservation method that involves altering the atmospheric environment around the food material (inside the package) by substituting the natural composition (approximately 78 % N_2, 21 % O_2) with single or a mixture of protective gases (for instance, 40 % CO_2, 60 % N_2) it creates an atmosphere that slows down the growth of microorganisms and the rate of oxidation (Church & Parsons, 1995).

The quality of food products greatly influences the purchasing decisions of consumers, who prefer fresh commodities over frozen or processed ones. At the same time, too little processing can enable deterioration due to microbial actions and various chemical reactions, leading to negative consumer responses and financial losses for manufacturers and producers (Osman Erkmen & Bozoglu, 2016). Regarding consumer demands, MA packaging aims to protect from microbiological and physiochemical alterations and keep sensory attributes of the food material. The effectiveness and composition of MA packaging depend on various factors such as the type of raw material, fish species, gas composition, storage temperature, filling degree (FD, ratio between product and package volume) and properties of packaging materials.

Due to all the aforementioned, the use of MA packaging for food preservation is extremely popular in a wide range of applications, including meats, poultry, fish, vegetables, fruits, pasta, cheese, bakery products, potato crisps, coffee and tea (Davies, 1995). Hence, the global importance of this technology is visible, particularly for the Norwegian seafood industry as a

significant exporter of fish products. The use of MA packaging can help extend the shelf life of these products, thereby reducing food waste, improving the quality of products for export, and expanding the feasible exporting distance.

By setting the package air composition, MA packaging can effectively inhibit microbial spoilage, reduce enzymatic reactions, and delay the onset of oxidative processes. This technology has been widely adopted in the food industry to maintain freshness and extend the shelf life of various perishable products, including seafood. This method can significantly reduce food waste and improve the availability and accessibility of fresh and high-quality food (Sivertsvik, Jeksrud, et al., 2002).

The gases commonly used in MA packaging are oxygen, nitrogen, and carbon dioxide. However, other gases such as argon or carbon monoxide are also in use (Mullan & McDowell, 2011). Still, oxygen and carbon dioxide are the key gases used in MA packaging due to their preservative effects on food products. Oxygen is introduced in MA packaging, in a limited range of products, to inhibit the growth of anaerobic bacteria and restrain undesired reductive reactions, as trimethylamine N-oxide (TMAO) to trimethylamine (TMA), this second one responsible for bad odour on fish (Hoel et al., 2022; Lerfall et al., 2022). However, it is still used mostly in low concentrations as it can lead to oxidative rancidity and browning and stimulate aerobic bacterial growth (Velu et al., 2013).

Carbon dioxide is mainly recognized for its antimicrobial properties as research has demonstrated that higher concentrations of CO_2 in the package lead to increased storage life at refrigeration temperatures (Rotabakk et al., 2006). However, at very high concentrations, it can have an unpleasable odour. It is soluble in the aqueous part of the product (water and lipids), forming carbonic acid (H_2CO_3), which reduces the pH and increases the acidity of the solution. This change in pH prolongs the lag phase of microbial growth, effectively lowering the growth rate throughout the logarithmic phase. However, the pH drop may be minimal, and there may not be a significant bacteriostatic effect (Velu et al., 2013).

On fish packaging, another drawback of CO_2 is the potential decrease in the product's water-holding capacity (WHC): fish muscle has a tendency to absorb a significant amount of carbon dioxide in the form of dissolved CO_2, as a result, the pH of the muscle decreases, leading to a reduction on the muscle proteins' ability to retain water (reducing the WHC). This issue can be addressed by including an absorbing pad, typically made of cellulose, beneath the fish product (Floros & Matsos, 2005). Thus, the final design of a MA packaging system for seafood, with a conventional polymeric package (tray) and a film to seal the system, is represented by Figure 1.3.

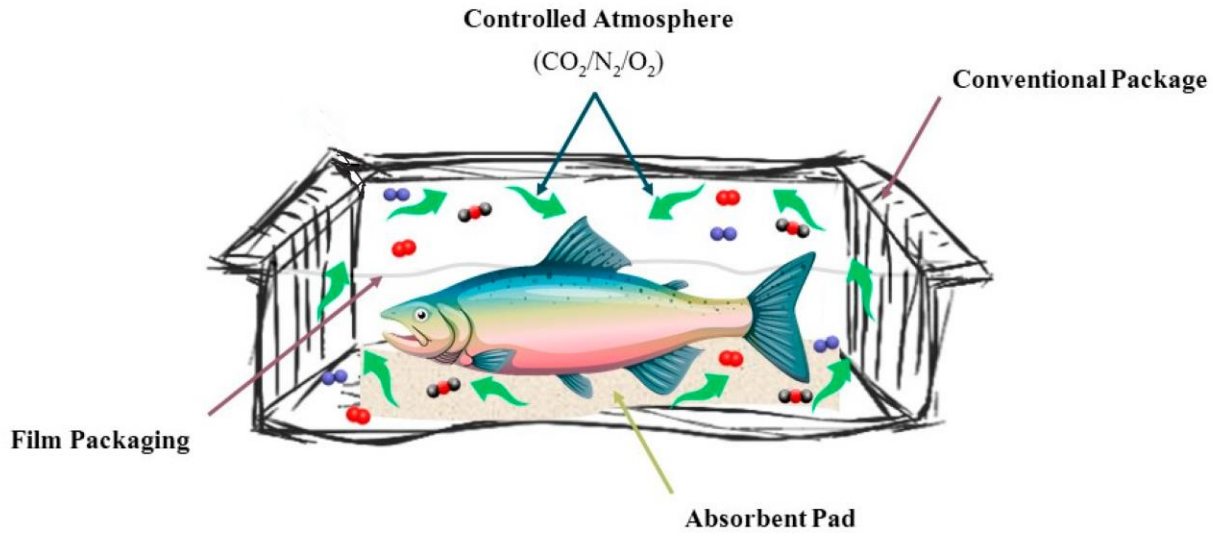

Figure 1.3- Schematic representation of MA packaging technology applied to fishery products. Adapted. Source: Amaral et al. (2021).

While there is no established optimum CO_2 concentration for maximum shelf life, increasing the initial amount (as the CO_2 packaged will be absorbed by the product until the equilibrium) of CO_2 generally extends the product's storage duration. However, above all constraints, CO_2 has a physical major upper limitation, the absorption of carbon dioxide by the food (e.g., fish muscle) can also cause a decrease in the internal pressure of the package. This leads to a decrease in the package volume due to the natural equilibrium of internal and external pressures as the packaging materials are not totally rigid, resulting in package fragilization and even collapse. Therefore, the upper limit of CO2 concentration is constrained by the FD and the flexibility of the packaging material (Rotabakk et al., 2006).

In order to mitigate the collapse problem while raising the CO_2 concentration on the package, nitrogen can be added to the gas mixture within the package and/or the FD can be reduced (Sivertsvik, Rosnes, et al., 2002). The incorporation of nitrogen helps maintain an appropriate internal pressure, preventing collapse and ensuring the integrity of the packaging (Floros & Matsos, 2005). Nitrogen is an inert gas, unlike CO_2, as it is hardly soluble in the food and it does not contribute to taste, colour, or odour to the packaged food. It plays a role in delaying oxidative rancidity and inhibiting the growth of aerobic bacteria in the absence of oxygen. However, nitrogen is not effective against anaerobic bacterial growth (Velu et al., 2013).

In the light of the CO_2-nitrogen-FD relation, a recommended filling degree of 25 % to 33 % is recommended for seafood as an optimization for package volume efficiency and still ensure the availability of bacteriostatic CO_2 and prevent package collapse. Nevertheless, low FDs, as recommended, are generally undesirable due to low packaging efficiency (Rotabakk et al., 2006), so another solution to the matter was necessary.

Soluble gas stabilization (SGS) is a new process involving dissolving CO_2 into the food product before it is packaged, i.e., a MA packaging pre-step to overcome the solubility issue and enhance the effectiveness of MA packaging. As illustrated by Esmaeilian et al. (2021) for a salmon fillets processing line (Figure 1. 4), the fillets pass through a chamber in which the atmosphere inside is substituted by 100 % CO_2 showing the dissolution of CO_2 into the product, specifically on the liquid phase, and then packaged in MA. The solubility of CO_2 increases at lower temperatures and higher pressures, allowing for sufficient CO_2 to be dissolved into the product during a short period in pure CO_2 before retail packaging. This process takes advantage of CO_2's bacteriostatic effect and pH-reducing properties (Sivertsvik, 2000).

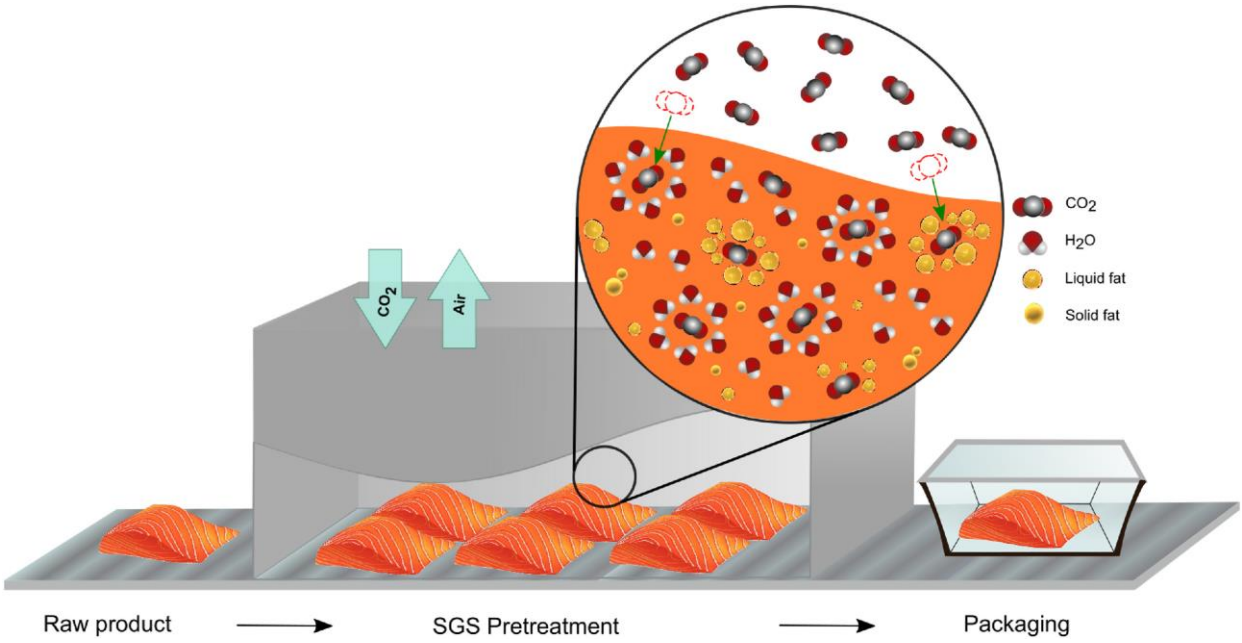

Figure 1.4. SGS process in a production line and CO_2 dissolution mechanism into the product. Source: Esmaeilian et al. (2021).

SGS offers several advantages over traditional MA packaging. Firstly, by dissolving CO_2 directly in the food, this technology ensures a high concentration of this molecule within the food material itself. This not only prevents the absorption from the MA, and therefore the pressure/volume decrease, but also allows high filling degrees, improving packaging efficiency without compromising the quality of the packaged food products (Rotabakk et al., 2006). Secondly, it enhances the preservation capabilities of MA packaging by making possible an MA with a higher concentration of CO_2. This increases the effectiveness of the MA in inhibiting microbial growth and reducing the oxidation of food products, thereby extending the product's shelf life. Thirdly, SGS can be applied to a wide range of food materials, including liquid and solid products. Its versatility makes it a viable solution for various food industries. Lastly, SGS has shown promising results in maintaining the sensory attributes and nutritional quality of the packaged products, making it an attractive option for food manufacturers aiming to deliver high-quality and fresh products to consumers (Esmaeilian et al., 2021). Overall, SGS well

addresses the solubility challenge associated with CO_2 in MA packaging. This innovative approach contributes to the efficiency and sustainability of packaging in the food industry.

This dissertation thesis aimed to investigate the benefits of SGS for preserving fish products, more specifically commercial Norwegian fish cakes (pre-cooked minced fish mixed with starch and seasonings in patties shape), considering the timing of application for heat processed products, as CO_2 solubility phenomena is highly impacted by temperature.

The work focused, firstly, on examining the solubility of CO_2 in fish cakes, which are pre-cooked before packaging. In this context, the aim was to compare the technology application just after the cooking while the product is still hot or when the product is already cold. This is possible by a gravimetric method, where it is measured the buoyancy force of a submerged package, which its directly related to its volume in each measuring point - changed through time due to CO_2 dissolution. The evolution of the buoyancy force, the product's Henry's constant (obtained through the ideal gases law) and the final concentration of CO_2 on the package headspace enables the calculus of the absolute amount of CO_2 in the product provided by the technology. In addition, the effect of pre-dissolved CO_2 on the overall quality of pre-cooked fish cakes was investigated during storage at 4°C, including pH, drip loss and texture assessment. The ultimate goal was to determine the effect of the treatment on the shelf life, indicated by the microbial growth on the product over time. The CO_2 pretreatment was conducted, exposing hot and cold fish cakes to 100 % CO_2 for 0.5h, 1h and 1.5 h at 4°C (the Control group was kept in air at 4°C) before repackaging in 40 % CO_2, 60 % N_2 MA and storage for 40 days. As the first microbiological test did not produce statistically valid results, a second experiment was designed without heating and with a longer exposition to the treatment. Thus, exposure times of 1h and 18h at 4ºC (the Control group was directly packaged in MA). Foreseeing an expansion of the package volume due to desorption after such a long exposition, a 70 % CO_2 MA group was added.

1.2 Contribution of the author to the work

All the work detailed in this thesis was performed by its author during a research stay of six months at NTNU, besides the execution of the packaging part of experiments 1 and 2. In other words, from the provided material to the packaging on MA, I had help from colleagues due to the high number of samples and limited time window available.

1.3 Organization of the dissertation

This thesis is divided into 7 main chapters, for which a brief description follows.

Chapter 1 presents an overall perspective on the subject of the dissertation, and sets the foundations for an understanding of the following chapters. It provides a short overview of the problem under study, the main methods used, and the main goals of the work developed.

Chapter 2 presents a literature review related to the theme under study, providing context for the current work by quoting previously published results and setting a theoretical basis for the following chapters. In the first section of this chapter, the current problem of the demand for as minimally processed foods at the same time of extension of shelf life, more critical on fish, is discussed and the need for novel food processing technologies is explored. Next, the potential of SGS process as an answer to the challenge is assessed. The third section approaches the effects of SGS specifically in seafood. Last, the combination of SGS and heat treatments, by analyzing their effect on each other efficiency and joint effect is discussed.

Chapter 3 presents all materials used in this work, as well as a detailed description of all procedures employed. A description of all experiments, their design, factors and sample preparations is provided. The methods used for CO_2 solubility study, microbiologic growth, pH, texture, drip loss determinations are also elucidated in a detailed manner. Finally, the statistical analysis employed is described.

Chapter 4 contains all results obtained throughout the work developed, as well as a detailed discussion and comparison to data available in the literature. The results obtained through buoyancy of MA packages are presented, and the compared evolution of CO_2 absorption is described. Then, the experimental results obtained from the storage experiment are presented - namely microbiologic growth, pH, texture and drip loss. The microbial growth results were inconclusive, so a new experiment was proposed, and the new results were evaluated. A joint analysis of the quality parameters was made to conclude on the overall shelf-life and quality effects.

Chapter 5 presents the main conclusions of the study.

Chapter 6 presents an assessment of the work developed, followed by suggestions for future work.

Chapter 7 lists all bibliographic references used in the preparation of this thesis.

2 Context and State of the art

The food industry is interested in developing solutions to increase product shelf life while preserving nutritional value and assuring safety. There is a variety of processing procedures with the potential to increase the product's shelf life, thermal and non-thermal ones (Esmaeilian et al., 2021). However, consumers are increasingly seeking out minimally processed, safe and healthy foods. Shelf-life extension tendentially means an increase in processing. In this context, companies prioritize raising these processes' efficacy. An approach could be combining methods towards an optimally less invasive process. Since all methods have limitations, a combination can have synergy and overcome them (Osman Erkmen & Bozoglu, 2016).

As set, this variety of settled techniques are applied to control spoilage of food, and can be divided in thermal and non-thermal. Thermal ones such as sous vide, pasteurization, high temperature short time (HTST) are well-established, even familiar to the non-specialized public, as microwave (volumetric) heating (MVH) and ultra-high temperatures (UHT). They are most efficient for preservation by killing spoiling and pathogenic microorganisms but also most likely to reduce products' initial quality. On the other side, non-thermal methods are less invasive, but at the same time, their use without a combination of other processes is hardly effective: being likely to compromise the product initial aspect is implemented individually (Fellows, 2009). In other words, the more intensively the treatments are applied towards better safety, the higher the possibility of detrimental effects on other quality metrics. Among them, flavour, colour, odour and texture affect consumer acceptability. In general, each process has specific positive and negative impacts, as shown in Figure 2.1 for some of the most usual ones.

This inverse relationship of consumers for minimally processed foods x safety and shelf life is even more critical when it comes to fresh fish: a very perishable food due to high water activity, neutral pH and nutrient-rich content of the flesh, besides the heavy microbial load on the surface (Tsironi & Taoukis, 2018). Marine fauna products are so sensitive that they need to be chilled or even frozen right away after being harvested in order to prevent microbial growth and quality degradation. Post-harvest processing techniques often seek to extend fresh fish's shelf life in order to increase commercialization and maximise resource use. Compared to other goods, they show a series of aspects that hampers traditional strategies for preserving seafood:

- freezing causes unwanted water loss (Jessen et al., 2014),
- chilling is less efficient as their natural microflora is more adapted to lower temperatures (Jessen et al., 2014),
- drying is trivially a bad option as initially it is not desirable to lose water (Hall, 2010),

- salting, a classic strategy, causes water loss and is not a consumer preference nowadays, due to health issues (Carlucci et al., 2015),
- limiting oxygen by modified atmosphere (MA) may reduce sensorial aspects (Debevere & Boskou, 1996),
- packaging in high oxygen concentration MA promotes fat oxidation beyond the general growth of aerobic bacteria (Guyon et al., 2016).

As demonstrated, fish exhibits notable detrimental changes to the majority of first approaches aimed at food preservation in general. Other products are more stable as they don't present the factors that enable microbial growth: grains and bakery products have low water activity, beverages have low pH, fruits and vegetables have on-harvest preserving processes, land animals' microbiota is not adapted to the cold, etc. Therefore, it is clear that fish, more than any other product, needs more sophisticated preserving techniques beyond the optimized use of the classic ones.

When seeking optimization of methods to navigate through the quagmire of food preservation, it's common to have as a basis the concept of Hurdle technology. This concept involves combining various existing and innovative techniques to create a series of barriers, or hurdles, that maximize microbial lethality while minimizing negative impacts on sensory and nutritional quality (Leistner & Gorris, 1995). These hurdles include factors such as temperature (high or low), water activity (a_w), acidity (pH), redox potential, preservatives (e.g., nitrite, sorbate, sulphite), and competitive microorganisms (e.g., lactic acid bacteria), which can be applied together, in a joint effect on food preservation, as illustrated with an example of combination for fish by Leistner and Gorris (1995) in Figure 2.2.

Although numerous hurdles have been identified to improve the stability and quality of food products, the list is far from exhaustive, with over 60 potential hurdles described thus far (Leistner, 2000). The selection and application of specific hurdles can occur either simultaneously or sequentially, depending on the nature of the hurdle and the overall processing requirements (Leistner & Gorris, 1995).

The implementation of hurdle technology, also known as combined processes, combination preservation, or barrier technology, has proven to be highly successful. By intelligently combining hurdles, this approach ensures microbial stability, safety, and the preservation of sensory, nutritive, and economic properties in food products (Leistner & Gorris, 1995). However, it is important to note that the utilization of hurdle technology may still result in the development of entirely new sensory characteristics for the product (Tsironi et al., 2020).

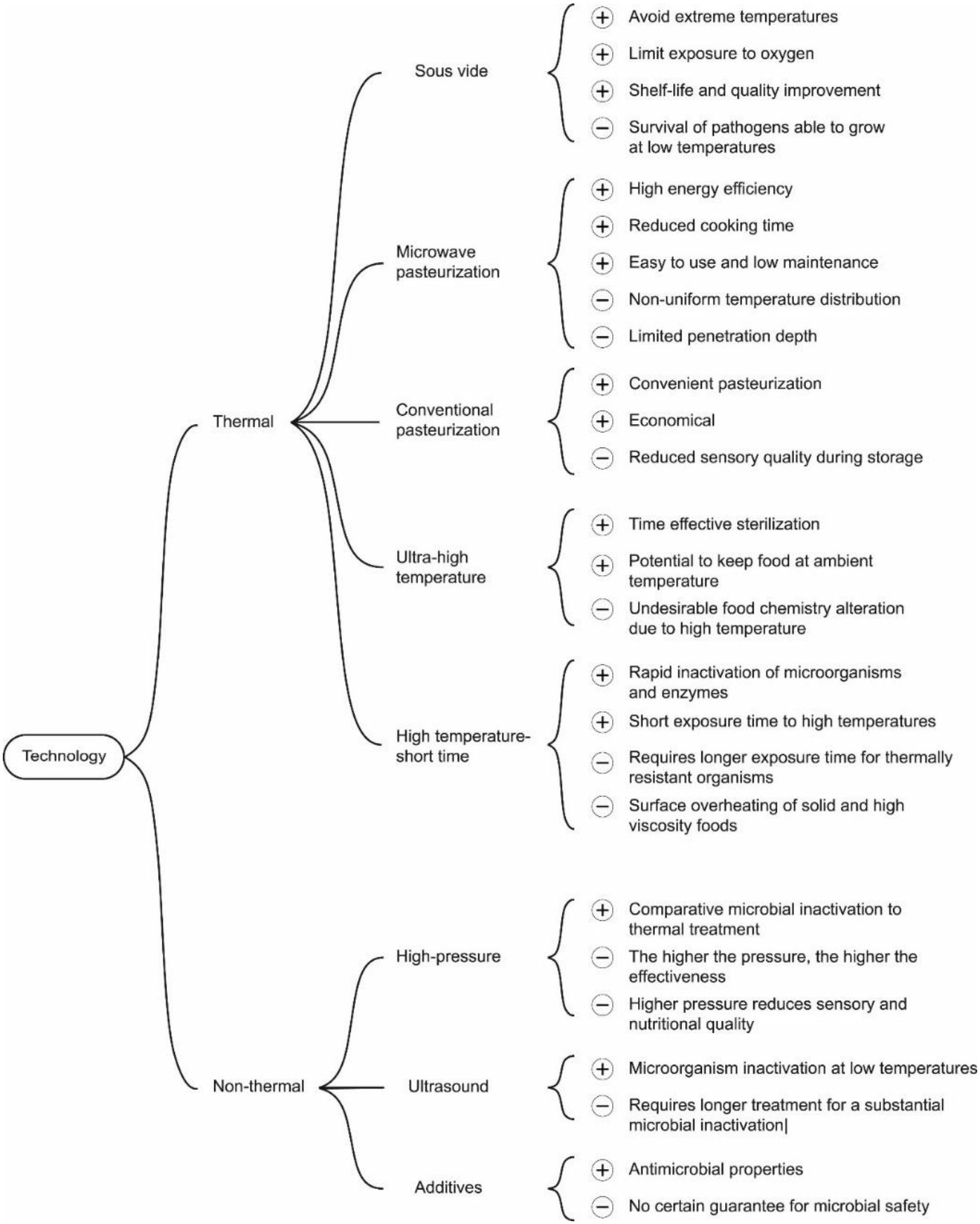

Figure 2.1. "Summary of the positive (+) and negative (-) traits of the thermal and non-thermal technologies" discussed by Esmaeilian et al. (2021), commonly used by the food industry. Source: Esmaeilian et al. (2021)

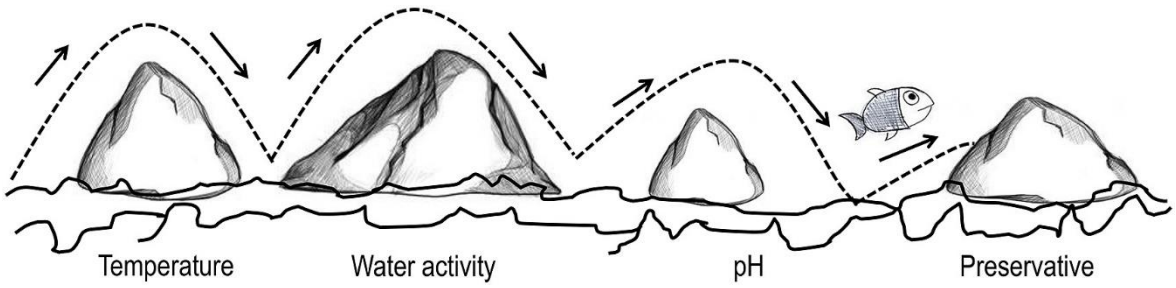

Figure 2.2. A possibility of hurdle combination for fish preservation. Source: Leistner and Gorris (1995).

In this enormous number of possibilities, it is crucial to choose the ideal synergetic hurdles for a given food product. For example, taking into account that bacteria are more resistant to heat at low a_w values and less resistant to heat when in the presence of preservatives. Additionally, fermented meat-based products can be classified as stable and safe provided both the a_w and pH levels are in a range outside the microorganisms' adaptations. The ability to further maintain quality and prolong the shelf life of food products is evidenced by the combination of traditional hurdles with new ones (Bozoglu & Erkmen, 2016).

For perishable products such as fish, lowering the temperature is the main and often single hurdle in use. However, this hurdle collapses if fish is exposed back to inappropriate temperatures during distribution and storage, leading to quality degradation and increased safety risks, such as *Vibrio parahaemolyticus* growth in oysters and histamine poisoning for scombroid fish, which both have a strong correlation with higher storage temperatures (FAO/WHO, 2011, 2013; Tsironi et al., 2017).

2.1 Soluble Gas Stabilization

Food preservation involves exposing microorganisms to an unfavourable environment in order to restrict their development, reduce their survival time, or even bring them to death. Microorganisms' potential responses to this harsh environment determine whether or not they will survive and grow. SGS technology was developed by Sivertsvik (2000), as a process of dissolving CO_2 into food, firstly seafood, prior to the packaging, so the product would already have CO_2 within when packaged, instead of only absorbing the CO_2 from the MA (Sivertsvik & Jensen, 2005). The basis for this technology is the antimicrobial action of CO_2, which limits microbial growth and other food-degrading mechanisms (Rotabakk & Sivertsvik, 2012), and this effect is positively correlated to the amount of CO_2 dissolved in the food matrix (Devlieghere et al., 1998). This is because when CO_2 dissolves in water, carbonic acid is created, then splitting into bicarbonate and hydrogen ions and lowering the pH of the solution (Chaix et al., 2014). In terms of production line efficiency, the process is viable as a sufficient amount of CO_2 can be dissolved into the product for up to 1-2 hours in pure CO_2 due to the rise of CO_2 solubility at lower temperatures and higher pressures (Rotabakk et al., 2006; Sivertsvik, Jeksrud, et al., 2004). At laboratory scale, SGS has shown to enhance shelf life and maintain food quality for

longer, with very little alteration in sensorial aspects (Mendes & Gonçalves, 2008; Rotabakk et al., 2008). The transportation of fresh goods over large distances is satisfied by longer shelf lives, which also helps associated sectors expand into new markets in the future. Additionally, it helps to ensure a reliable supply of food by lowering food loss along the food supply chain. Considering the world's rising population and rising food demand, one of the biggest difficulties facing the world is ensuring a safe, sufficient, and secure nourishment (Esmaeilian et al., 2021).

Moreover, SGS also improves MA packaging by offering a better degree of filling, resulting in higher storage and transportation efficiency. The issue with increasing the FD in traditional MA is the need for a greater CO_2 partial pressure, this is a higher CO_2 initial concentration on the MA, to dissolve the same amount of CO_2. As a result, the gas volume would be reduced further and the package would collapse. SGS overcomes the conflict between the amount CO_2 on the MA composition and specified FD and by dissolving CO_2 into the product prior to packaging (Cavalcanti et al., 2022; Rotabakk & Sivertsvik, 2012). In that regard, SGS improves packaging efficiency, lowering costs in logistics and materials, and reducing the quantity of plastic used in packaging, thus increasing sustainability in food manufacturing. Hence, adopting SGS treatment as an alternative to combine with traditional processes to reduce their intensity could be a sustainable decision when considering a hurdle strategy (Esmaeilian et al., 2021).

2.2 Effects of dissolved CO_2 and SGS on the quality and shelf life of aquatic food

Various metrics influence fish quality and shelf life: colour, microbiological load, adenosine triphosphate (ATP) degradation, texture, drip loss, etc. Thus, regarding the specificity of the processes' consequences, SGS effects also vary depending on what is applied together to the product. The first information consumers get on buying fish is the visual aspect, and myoglobin, responsible for fish's reddish colour, may oxide into metmyoglobin under high concentrations of CO_2, leading to flesh darkening. It has been confirmed for Atlantic salmon (Salmo salar L.) fillets, vacuum packaged samples presented a lighter and less yellowish and reddish colour by Chan et al. (2021) while denied by Jakobsen et al. (2022). This more recent study also found that the SGS technology had antimicrobial effects on the fillets.

Its known also that microorganisms are the main cause of fish spoiling and safety concerns. In this aspect, CO_2 addition lengthens the lag phase and generation time of spoiling and pathogenic microorganisms (Daniels et al., 1985) and delays their recuperation from trauma (Rode et al., 2015). The greatest specific growth rates (max) for Gram-negative spoilage bacteria were shown to present a negative linearity with the food matrix's CO_2 concentration level. Dissolved CO_2 had a greater impact on Gram-negative bacteria's growth parameters (max and lag phase) than on Gram-positive bacteria (Devlieghere & Debevere, 2000). However, it was shown that

Gram-positive bacteria like *Brochothrix thermosphacta* were inhibited. Because CO_2 tolerance varies between and among species, using SGS or MA packaging will change the original microbiome (Kolbeck et al., 2020). Therefore, SGS would slow bacterial growth by increasing the CO_2 concentration in the marine product (Sivertsvik & Birkeland, 2006). Despite all advantages, a critical consequence of CO_2 application was found in virulence and formation of toxins by regulatory stimulation of metabolic enzymes for particular infectious agents like *Helicobacter pylori* and *Citrobacter rodentium* (Park et al., 2011; Yang et al., 2009).

From the perspective of flavours, ATP-related compounds are impactful for the freshness and flavour of seafood (Hong et al., 2017). After animal death, ATP degrades sequentially into adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx). Besides, endogenous enzymes are primarily responsible for ATP degradation, bacterial enzymes can also cause HxR hydrolysis of HxR into Hx (Surette et al., 1988). Specifically Hx contributes to the gradual loss of pleasant tastes in storage muscle goods, while IMP is associated with desirable flavours (Hong et al., 2017), i.e., the lower the Hx formation, the higher IMP presence, the better the taste. It was reported that fish kept in pure CO_2 had a minor Hx content than other samples stored in other air compositions (Warthesen et al., 1980). Herring samples presented lower Hx accumulation kept under (60 %) CO_2 in comparison to those stored in ice (Özogul et al., 2000). Salmon fillets also presented lower Hx concentration when treated by SGS (Jakobsen et al., 2022). Concordant findings were seen in 40/45 % CO_2 MA for deep-water pink shrimp (Gonçalves et al., 2003) as well as 60 % CO_2 MA for hybrid striped bass strips (Handumrongkul & Silva, 1994), which had both reduced Hx formation compared to those packed in air. These results can imply that the antimicrobial effect of CO_2 leads to minor accumulation of Hx as it is also due to bacterial activity (Gonçalves et al., 2003; Lerfall, Bjørge Thomassen, et al., 2018). Nonetheless, this is a tendency rather than a rule, as it is not true for all concentrations or all products: for instance, in sole fillets there were no significant alterations in the ATP breakdown products in CO_2 between percentages of 20 and 40 (López-Gálvez et al., 1998).

Despite some findings indicating SGS has little impact on the texture of food (Mendes & Gonçalves, 2008; Sivertsvik & Birkeland, 2006), others have found a negative relationship between pH and fish muscle firmness (Hyldig & Nielsen, 2001) and CO_2 causing a reduction in pH has been documented for farmed cod (Sivertsvik, 2007). Moreover, the decreased pH by CO_2 presence in fish's aqueous phase promotes expressive loss of the WHC and, consequently, loss of succulence (Sivertsvik, Jeksrud, et al., 2002). A hypothetical justification for the different results is based on the limited diffusion of the pH change on the product surface to the interior muscle (Mendes & Gonçalves, 2008).

A last important aspect, exposition to high CO_2 concentrations is found to raise the drip loss in fishes such as Atlantic salmon (Randell et al., 1999) and Atlantic cod (*Gadus morhua*) fillets (Sivertsvik, 2007). Furthermore, by snugging down, the package's headspace volume reduction may lead to increased drip loss (as the package's partially flexible walls contract, squeezing the product) (Rotabakk et al., 2006). SGS would prevent this effect (Rotabakk et al., 2008) as investigations demonstrated a reduction in drip loss for SGS-treated halibut (Rotabakk et al., 2008) and ready-to-eat shrimp (Sivertsvik & Birkeland, 2006).

Taking into account the aforementioned, the investigation into the effects of extra dissolved CO_2 on food, by SGS, on food has generally revealed intricate dynamics that contribute minimally or negatively to texture (firmness) and positive colour modifications.

2.3 SGS in combination with thermal processes

Sous vide is a cooking technique conducted with heat-processing in a vacuum plastic bag at accurately regulated duration and temperature, this last one being lower than 100°C as the bag is submerged in water for heat transfer. Its application on seafood can provide extended shelf life and consumer interest by facilitating cooking in a wide range of products (Coşansu et al., 2022). It is possible to sous vide have synergy to SGS considering the existence of organisms resistant to CO_2 action and to anoxic environment, but not to moderated temperature variations, so heat exposition on thermic treatments has a crucial function in the suppression of those (Stiles, 1996). Moreover, neither colour, drip loss or texture were expressively affected when the methods were combined compared to the single use of heating (Abel, Rotabakk, & Lerfall, 2019). The complicator factor on this approach is the gas layer in the closed bag that can be formed during cooking, related to lesser CO_2 solubility with increasing temperature leading to desorption, reducing heat transfer by acting as an isolator, and finally compromising antimicrobial effect (Abel, Rotabakk, Rustad, et al., 2019). Still, the final bacterial amount was lower than solo heating, so the CO_2 positive impact is stronger than the reduced heat transfer negative one, so focusing on countering the gas layer formation, a bigger outer pressure could oppose the inner one caused by the desorpted gas and minimising this phenomenon (Esmaeilian et al., 2021).

Differently to sous vide and conventional heat transfer, microwave volumetric heating (MVH), called this way because radiation enters the matrix directly and produces heat through the product's volume (Zhu et al., 2007), became attractive for industries by its greater energetic efficiency, short processing time, enhanced product properties, convenient utilization and minimal need of maintenance (Chandrasekaran et al., 2013). The weakness of this technology, as consumers also know by having microwave ovens at home, is the non-uniform heating and short penetration of this class of electromagnetic radiation. For stored salmon

loins, the difference in colour and the overall sensorial acceptance was very little between the MVH-SGS and MVH, whereas the antimicrobial effect was highly enhanced by SGS addition, with lower populations on short duration storage and a delay in reaching maximum final counts on long duration (Lerfall, Jakobsen, et al., 2018).

From an industrial standpoint, traditional pasteurization using an autoclave is a cost-effective and convenient technique. Low pressures can cause a food-package dead space during the heat treatment without an autoclave, which leads to product insulation. Autoclave offers a temperature distribution and a counter-pressure that act significantly better at relatively lower temperatures (Skipnes, 2014). The created counter pressure has a substantial impact on heat transfer if the application of SGS is made prior to the heat treatment (Rosnes & Skipnes, 2017).

Lerfall, Jakobsen, et al. (2018) identified that SGS had no effect on the stiffness and drip loss of vacuum-packaged pasteurized Atlantic salmon loins. Additionally, there was no discernible variation in colour between SGS and just vacuum samples in metrics of L*a*b* (a colour space system where L* for perceptual lightness and a* and b* for the four unique colours of human vision: red, green, blue and yellow). The loins with pre-dissolved CO_2 were, however, less reddish (lower a*-value) than those wrapped in vacuum alone, according to the Duncan comparison test. Nonetheless, analysing the reflective property of vacuum loins, it turned out that the treatment leads to more reddish tone, preferred on consumers vision. On sensorial aspects, SGS had presented a negative effect on the tenderness and juiciness of the fillets, which goes against costumers preferences in terms of meat quality (Font-i-Furnols & Guerrero, 2014). This effect may be explained not directly due to SGS process but by the lowered pH, which is known to make fish drier, firmer and harsher (Hyldig & Nielsen, 2001). Moreover, a jury scored SGS samples 4.4 versus 4.7 non-SGS ones, out of 9, a close difference Finally, SGS once more showed effective inhibition on the microbiota, blocking *Aeromonas spp*. recovery and viability.

When packed aseptically, the product can be sterilized using UHT, about 125°C for 1-5 seconds, resulting in a microbially stable product at room temperature (Datta & Deeth, 2001; LORENZEN et al., 2011). In addition to having a minimum shelf life of six months without requiring refrigeration, UHT foods maintain a high quality that is comparable to frozen and chilled meals. However, certain foods' chemistry may change unfavourably as a result of the elevated temperature employed during UHT (Fellows, 2022). Vianna et al. (2012) observed that both standard UHT milk and UHT milk treated with CO_2 exhibited lower pH while storaged, indicating the production of acids through various reactions such as the Maillard reaction, lactose breakdown, casein dephosphorylation, and proteolysis (Gaucher et al., 2008). Compared to UHT milk alone, UHT milk treated with CO_2 showed lower levels of rancid flavour and bitter taste (Vianna et al., 2012). The addition of CO_2 before UHT processing improved the product quality

by inhibiting microbial growth and addressing sensory defects associated with bacterial activation (Datta & Deeth, 2001).

Although UHT processing can partially inactivate microorganisms and enzymes, it may not be effective against heat-resistant organisms, affecting the shelf life of the product (Fellows, 2022). Nevertheless, several studies suggest that CO_2 treatment can present an antibacterial effect on psychotropic bacteria, including those producing heat-resistant extracellular enzymes, e.g., milk proteases (Ma et al., 2003; Martin et al., 2003). The stability of these proteases can constrain product quality and shelf life, as they can cause age gelation by proteolysis (Datta & Deeth, 2001). Vianna et al. (2012) demonstrated that standard UHT milk presented higher protease activity from microorganisms, resulting in increased proteolysis compared to UHT milk with CO_2. Therefore, CO_2 treatment can improve the physicochemical aspects of UHT products through microbial and enzymatic inhibition. Additionally, the bacteriostatic action of CO_2 can be enhanced at higher temperatures (Erkmen, 2000), indicating a convergence of SGS and heat against bacterial thrive.

A last conventional one, HTST pasteurisation may mitigate or reduce negative quality changes caused by the thermic process by rapidly inactivating organisms and enzymes with temperatures of 72-75°C within 15-30 seconds (LORENZEN et al., 2011; Ohlsson & Bengtsson, 2002). Nevertheless, in an effort to limit the quantity of spoiling organisms, particularly heat resistant genera, HTST and holding durations frequently go above what is necessary for legal pasteurisation (Loss & Hotchkiss, 2002). Additionally, due to superficial overheating, HTST is ineffective for some products, such as solid and highly viscous foods, which can outweigh its benefits (Ohlsson & Bengtsson, 2002).

Microorganisms' tolerance to heating tends to rise along with incubation temperature, particularly true for spore-forming organisms (Jay et al., 2008). Therefore, raising heating until it reaches the necessary degree of bacterial suppression seems neither logical nor cost-effective. Hence, the shelf life of milk products subjected to HTST may be shortened by the recurrence of a substantial amount of spore-forming bacteria during pasteurisation (Meer et al., 1991). (Loss & Hotchkiss, 2002) found that *Pseudomonas fluorescens* R1-232, among the top prevalent milk spoiling microbes, and *Bacillus cereus* ATCC 14579 spores, a heat resistant bacteria, had lower heat survival rates for milk with previous dissolved CO_2. Additionally, milk heated between 67-93 °C and subjected to treatment with a range of CO_2 levels, varying from 44 to 58 mM showed a substantial decrease in the number of surviving standard plate count (SPC) microorganisms. Therefore, it is possible to use CO_2 as a process catalyst to make microorganisms more sensitive to heat, hence improving microbial inhibition in pasteurisation.

Based on the preceding discussion, it is visible how SGS is a promising technology and very suitable as a hurdle together with other processes, including thermal ones, to answer the

challenges of preserving food and mainly fish products. The effects of dissolved CO_2 by SGS are not always fully beneficial; nonetheless, studies through the last two decades show it is much more likely to be a worthy technology to include in heated or fresh products. Still, regarding the synergy between CO_2 treatments (SGS) and heat processes, no study addresses the question of when in the production line of heat-treated products is optimal for the process inclusion (either on fish cakes or similar products). Thus, the work carried on this dissertation thesis aimed to extend the knowledge on heat treated seafood by establishing new knowledge of solubility of CO_2 in fish cakes regarding high temperature difference due to treatment as heat counteracts the solubility of CO_2 and evaluate the general quality and microbiological growth of fish cakes combining pre-dissolved CO_2 and MAP during cold storage.

3 Materials and Methods

The experimental setup was divided into two experiments. Experiment 1 (Figure 3.1) was designed to study the solubility of CO_2 in fish cakes. Experiment 2 (Figure 3.2) was a storage experiment to follow the effect of pre-dissolved CO_2 on quality attributes (physicochemical aspects and microbial stability) of MA packaged fish cakes with the same factors as Experiment 1. After inconclusive results on the microbial stability, Experiment 3 (Figure 3.3) was designed with different factors specifically to cover this missing information.

Experiments were performed using commercial Norwegian fish cakes. In Experiments 1 and 2, the experimental factors were the process' initial temperature (immediately after heat treatment or after 2h chilling until 4°C), SGS processing time (0.5, 1 and 1.5 h), and storage time (0, 8, 16, 24, 32, 40 and 72 days). These experiments were performed in two rounds, separated based on the process's initial temperature (n = 54 + 18 for the first round and n = 72 + 24 for the second one).

In experiment 3, no heat treatment was applied to the fish cakes, and the experimental factors were SGS processing time (1h and 18h), MAP composition (40 % CO_2, 60 % N_2, and 70 % CO_2, 30 % N_2), and storage time (0, 6, 15, 34, 41, 48 and 55 days). The experiment was performed in two rounds, separated based on SGS processing time since it was applied an overnight treatment, being only possible to continue on the following day (n = 48 for both rounds).

3.1 Ordered material

Commercial fish cakes (Norwegian *Fiskekaker*, 25 kg) were purchased from Domstein Sjømat AS distributor (Trondheim, Norway), who distributes for Engerviks Fabrikker AS (Lindesnes, Norway). The fish cakes were transported in 2.5 kg polyethylene bags in 5 kg cardboard boxes to the Norwegian University of Science and Technology (NTNU, Trondheim, Norway). Upon arrival at NTNU, the material was storage in a 4°C fridge room for a day until the experiment execution.

3.2 Experimental designs

3.2.1 Experiment 1: Solubility of CO_2 in cold and hot fish cakes

In the first experiment, fish cakes were equally spread on metal trays heated in a steam cabinet (SelfCookingCenter®, Rational, UK) to a core temperature of 85°C. The first part (n = 18) underwent SGS process (CO_2 treatment, described subsequently) immediately after reaching 85 ºC, simultaneously as they were cooled down in a 4°C fridge with the aid of dry ice for 0.5, 1.0, and 1.5 h (n=6 for each group), respectively. The last samples (n=24) were cooled down in

the trays to 4°C (fridge temperature, 2h cooling time) with the aid of wet ice before being treated with SGS according to their hot equivalents (0.5, 1, and 2 h, n=6 for each group). In addition, a group of samples was stored in air before packaging (0 h SGS time) to simulate a standard commercial protocol. The SGS process and the air storage for the last group was followed by re-package in MA-trays with 40 % CO_2 and 60 % N_2 (Group IDs: Hot 30, 60, and 90, Cold 30, 60 and 90, and Control, respectively) by using a tray-sealing system, and an initial headspace atmosphere of 40 % CO_2 (40,5 ± 0.3 %) and balanced with N_2 and a FD of approximately 1/3.

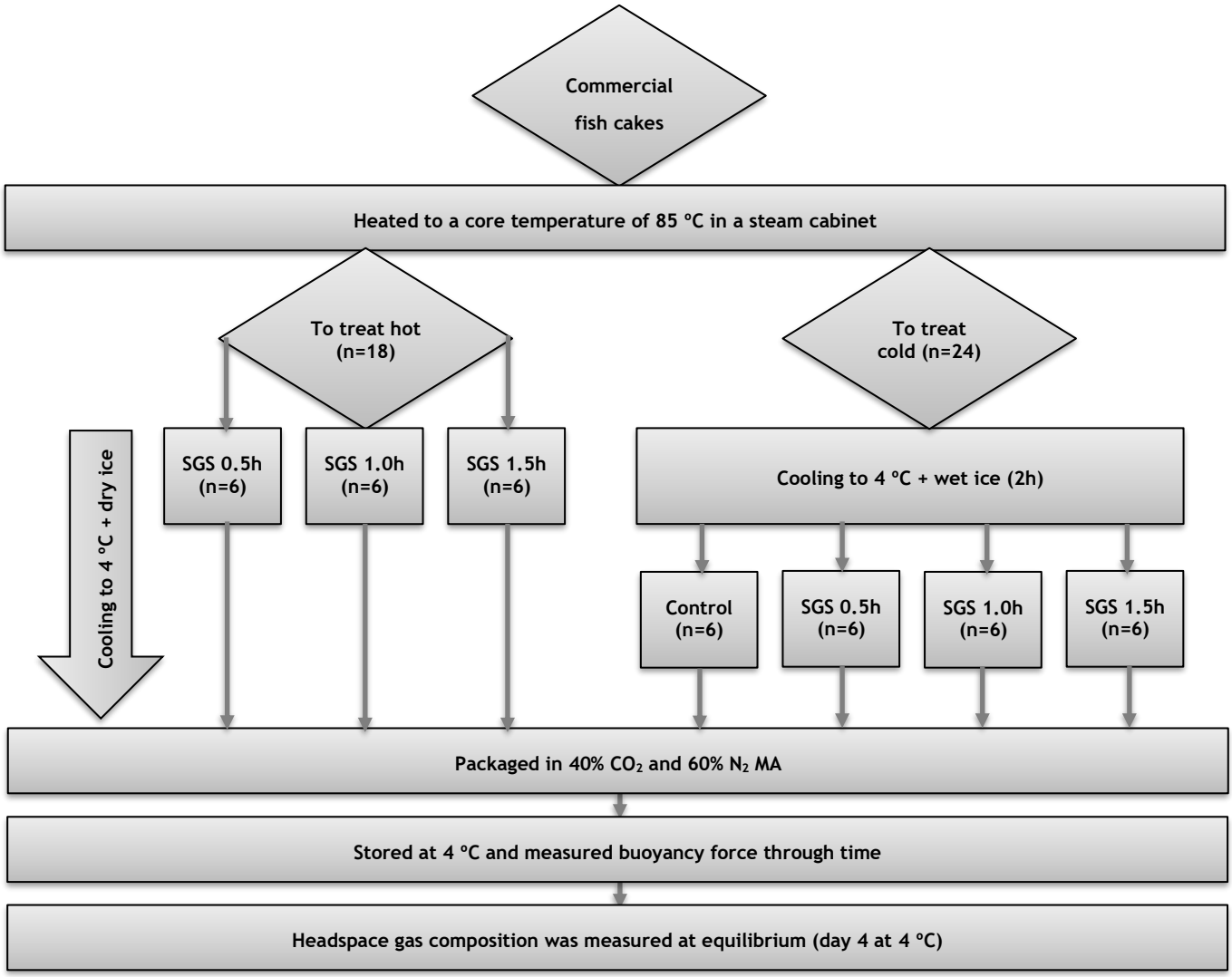

Figure 3.1- Experiment 1 design. SGS stands for soluble gas stabilization and MA stands for modified atmosphere.

The SGS treatment was performed according to a modified method described by Rotabakk et al. (2006) with 98.5 % food-grade CO_2. In the meantime, the Control remained in air. In sequence, each group was repacked into 215 ml semi-rigid crystalline polyethylene terephthalate trays (C2125-1A, Færch Plast, Denmark) with the aid of a tray sealing packaging machine (TL250, Webomatic, Germany). The initial atmosphere removed (final vacuum pressure of 25 mbar) and the filled with mix of gases chosen for the packages. The cover film

constituted a 40 mm mix of polyethylene, ethylene vinyl alcohol, polyamide, and polyethylene terephthalate (Topaz B-440 AF, Plastopil, The Netherlands). Food-grade CO_2 (40 %) and N_2 (60 %) were combined with the aid of a MAP Mix 900 gas mixer (Dansensor, Denmark). A sample FD of approximately 33 % (71 ± 2 g) was achieved and they were stored at 4°C for 3 days to reach the equilibrium in CO_2 between the fish cakes and the package's atmosphere.

The headspace gas composition was measured using a Checkmate 9900 oxygen and carbon dioxide analyzer (PBI-Dansensor, Denmark) as described by Abel et al. (2018). Packages without samples were used to confirm the if the samples were packaged in the selected MA on the packaging moment and in all trays at the end of the storage period (71 h). The headspace gas volume was again measured 2.5, 7, 11, 23, 35, 47, 59, and 71 h after packaging for all the samples, according to a method described by Rotabakk et al. (2007) and modified by Abel et al. (2020). The concentration of absorbed CO_2 was calculated according to Rotabakk et al. (2007) related to changes in package volume as described by:

$$C_{CO_2}^{t=\infty} = \frac{1000 \times P\left(V_g^{t=0} - V_g^{t=\infty}\right) \times MmCO_2}{R \times T \times W_f} \quad (1)$$

Where $C_{CO_2}^{t=\infty}$ is the total CO_2 (ppm) absorbed by the product, P is absolute pressure (Pa), $V_g^{t=0}$ is gas volume (m^3) at start, and $V_g^{t=\infty}$ at equilibrium, $MmCO_2$ is the molecular mass of CO_2, R is the ideal gas constant (in coherent units), T is the absolute temperature (K), and W_f is the mass of the product (kg).

According to Henry's law, the food matrix obtained equilibrium with the package's atmosphere when the concentration of CO_2 in the package's atmosphere is proportional to the concentration of CO_2 absorbed in the matrix. According to Schumpe et al. (1982) this can be described as:

$$P_{CO_2}^{t=\infty} = H_{CO_{2,P}} \times C_{CO_2}^{t=\infty} \quad (2)$$

Where $P_{CO_2}^{t=\infty}$ is the partial equilibrium pressure of CO_2 in the headspace gas (Pa), $H_{CO_{2,P}}$ is the temperature-dependent Henry's constant for CO_2 in the sample (Pa × ppm^{-1}).

3.2.2 Experiment 2: Effect of SGS-technology on quality of MA packaged fish cakes

In Experiment 2, the methodology was followed as described for Experiment 1, until the re-packing step with the difference in the number of samples per ID Group (n=18 instead). The experiments were conducted simultaneously and with the same experimental conditions. Three packages were opened and examined for physiochemical and microbiological parameters at 0, 8, 16, 24, 32, 40 and 72 days during storage at 4°C.

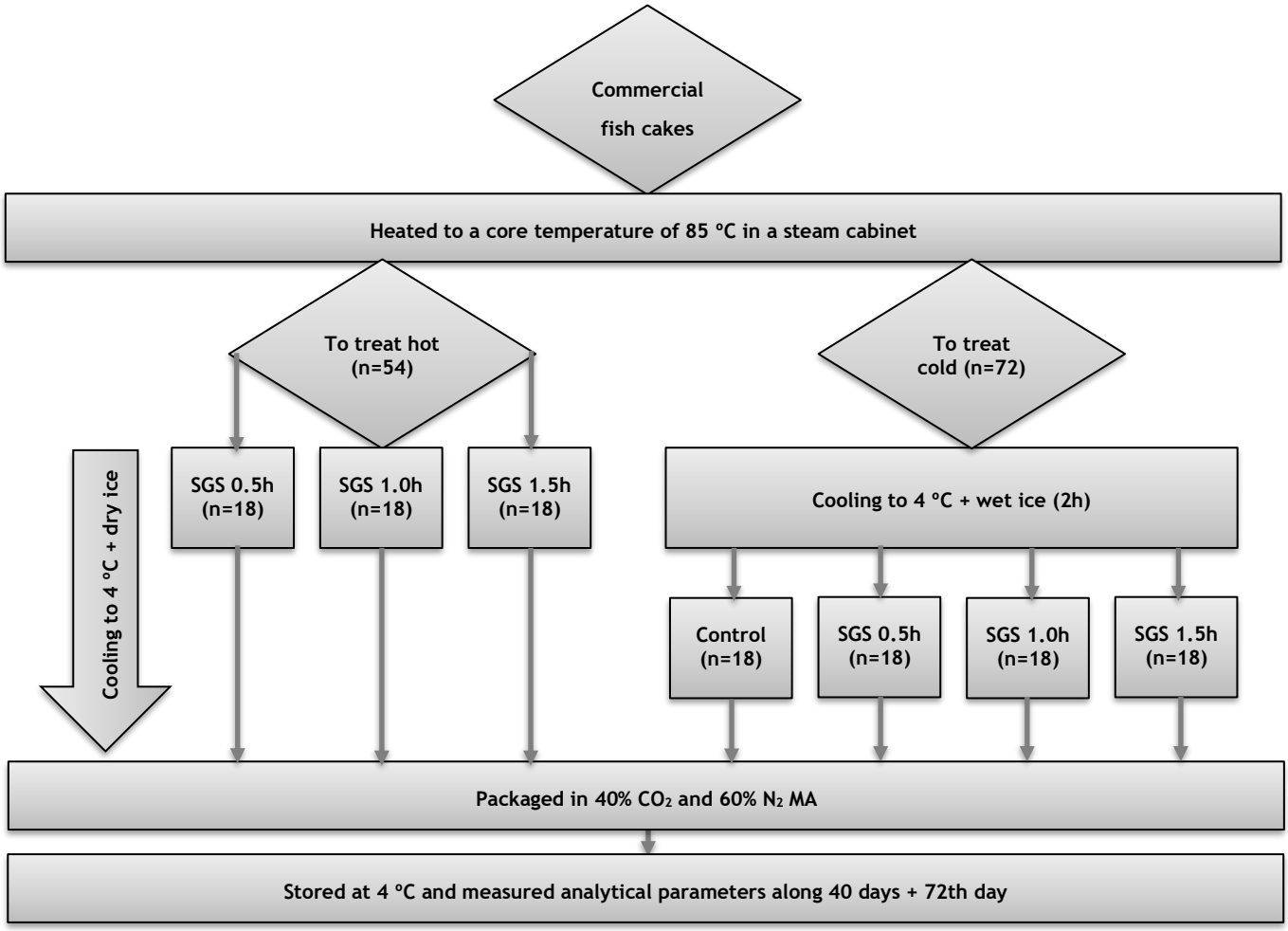

Figure 3.2 – Experiment 2 design. SGS stands for soluble gas stabilization and MA stands for modified atmosphere.

3.2.3 Experiment 3: Microbiological stability of SGS-technology on fish cakes

In the third experiment, fish cakes were immediately submitted to the SGS process without any heating. All the experiments were performed at 4°C. The first part (n = 36) was pretreated with CO_2 for 18 h (98 % CO_2), the second part (n = 18) was kept in the supplier's package for 17 h and submitted to the SGS process for 1h (98 % CO_2), in addition, a group of samples (n = 18) was kept in the supplier's package before repackaging (0 h SGS time). In sequence, all samples but half of the 18h group were repacked in 40 % CO_2 food-grade MA, in accordance with Experiments 1 and 2's repackaging step conditions. That other half was repacked instead in 70 % CO_2 and 30 % N_2, foreseeing CO_2 desorption on the first set MA regarding the long process (Group IDs: 18h-70%, 18h-40%, 1h-40% and Control-40%). Also, a different sample DF of approximately 47% (102 ± 2 g) was achieved and they were stored at 4°C. Three packages were opened and examined for microbiological assessment at 0, 6, 15, 34, 41, 48 and 55 days during storage at 4°C.

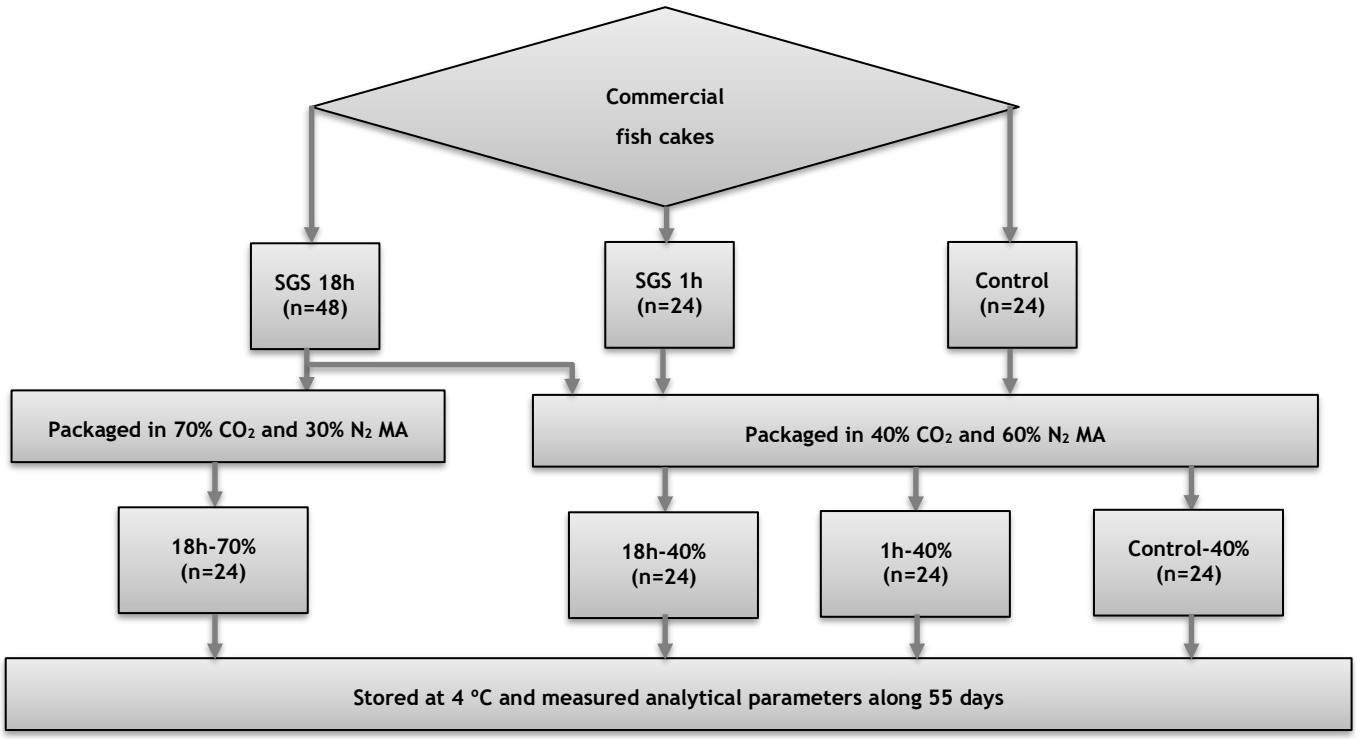

Figure 3.3 – Experiment 3 design. SGS stands for soluble gas stabilization and MA stands for modified atmosphere.

3.3 Microbiological analysis

A 10-g piece of fish cake was aseptically transferred to a sterile stomacher bag and diluted 1:10 with sterile peptone water (0.85 % NaCl and 0.1 % neutralized bacteriological peptone) and homogenized for 60 s using a Stomacher 400 lab blender (IUL Masticator, Spain). Appropriate serial dilutions were made in peptone water. Total aerobic plate count (APC), were quantified on Lyngby's iron agar (Oxoid) supplemented with 0.04 % l-cysteine (Sigma-Aldrich, Oslo, Norway), considering the possibility of H_2S-producing bacteria (black colonies). The plates were incubated at 22°C for 72 ± 6h.

3.4 Fish cake pH

pH was measured in the center of the fish cake at each sampling point using a Testo 206 pH2 - meter (Testo SE &Co, Germany). The pH meter was calibrated before use by a two-point calibration at pH 4 and 7.

3.5 Texture

Two texture aspects were measured, firmness and crust breaking force. Measurements were done using a texture analyzer (Stable Micro System Ltd, TA-XT plus, Godalming, UK) by pressing the center of the fish cakes with a 20 mm Diameter Cylinder Probe and measuring the counterforce. The fish cakes were impelled at a rate of 1 $mm \cdot s^{-1}$ until 75 % of the sample height. By the fish cakes production process (specifically the precooking of it before packaging), it

forms a crust layer on the outside, making the force to break this layer the biggest one, so the peak of the measurement curve. Also, the sample firmness was defined as the force at 60 % of the final penetration distance.

3.6 Drip Loss

The percentual drip loss could be calculated by weighting the absorbent pad of each sample package on the point of assessment. The difference from a dry pad is drip lost from the sample. With this, the drip weight can be divided by the respective sample weight in order to obtain its relative drip loss.

3.7 Statistics

Statistical analyses were carried with aid of Minitab 17.0 (Minitab, Coventry, UK). It included outlier test, analysis of variance (ANOVA), and general linear modeling (GLM). Outlier testing was carried using Grubbs outlier test at level $p < 0.05$. GLM was carried using Tukey's HSD test at level $p < 0.05$. In order to satisfy the requisite of equal variance and normal distribution, all statistical analyses of microbial stability were made on log-transformed data.

4 Results and discussion

SGS came as a solution for the snuggling down phenomenon by CO_2 dissolution. This enables packaging food in MAs with higher concentrations of CO_2 and/or DFs, therefore extending shelf life without critical issues while improving logistical and raw-material efficiency. To illustrate with actual images the gravity of the highlighted problem, below are photos from an extra experiment – which has further information on the appropriate section, 6.2 – comparing straight packaged in 20 % (Figures 4.1 and 4.3) and 98 % (Figure 4.2 and 4.4) CO_2 (not treated by SGS).

Figure 4.1. Side angle of fish cakes packaged in 20 % CO_2 MA.

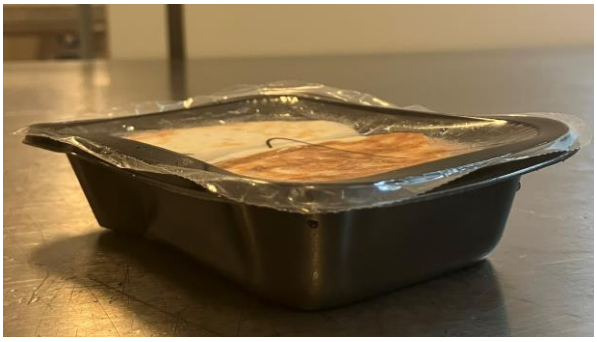

Figure 4.2. Side angle of fish cakes packaged in 98 % CO_2 MA.

Figure 4.3. Back of fish cakes packaged in 20 % CO_2 MA.

Figure 4.4. Back of fish cakes packaged in 98 % CO_2 MA.

One can clearly see a difference on the volume from CO_2 dissolution in food without pre dissolved CO_2. Figure 4.1 displays a normal package, with a noticeable distance between the package film and the fish cakes. This indicates that at a low CO_2 concentration, the snuggling down phenomenon is not observed and the fish cakes retain their original shape and structure – it is well-known as concentrations higher than this are applied nowadays in commercial products (Noseda et al., 2014). On the other hand, Figure 4.2 exhibits a snuggled-down appearance, with the fish cakes visibly compressed. The absence of a discernible distance

between the package film and the fish cakes indicates a significant snuggling down effect caused by the high CO_2 concentration.

Moving to the back view, Figure 4.3 portrays the back of packages without any signs of deformation, similarly to Figure 4.1, reaffirming the absence of the snuggling down phenomenon at a low CO_2 concentration. In contrast, Figure 4.4 showcases the back packages completely snuggled down, leading to squeezing of the fish cakes and compromising the integrity of the package. This visual observation further highlights the significant snuggling down effect that occurs when a higher CO_2 concentration is used for packaging.

Therefore, it is not possible to increase the CO_2 concentration on MA packages and extending the shelf-life without addressing this problem first. Therefore, the importance of SGS becomes of major concern. With this stated, optimizing its efficiency on the production line is necessary, empirically determining if it is more effective to apply the technology while the product is still hot or only after cooling – due to the SGS synergy with thermal treatments and CO_2 solubility, which is highly dependent on temperature.

4.1 Solubility of CO_2 in cold and hot fish cakes

The solubility of CO_2 depends on the product's composition (Abel et al., 2018; Sivertsvik, Rosnes, et al., 2004). This work considered negligible the water and lipid differences among fish cakes due to the high-quality standards of the preparation (the material came from the same batch). Yet, for validation purposes, the water and fat content that are indicated by the manufacturer (respectively 77.3 % and 3.3 %) were considered.

4.1.1 Henry's constant of CO_2 in 4°C fish cakes

The Control samples showed a Henry's constant (33.2 ± 1.8 Pa × ppm^{-1} at 4°C) very close to the water one, which, besides the product being 4/5 moisture, does not indicate a valid value by itself. Fish generally has a closer composition to the product than pure water, however, when compared to its Henry's constant value, they do not match, since it lies within 40 to 50 for fish (Sivertsvik, Rosnes, et al., 2004). Getting more specific on the product, a model seafood with recipe (Abel et al., 2018) quite similar to the fish cakes one (minced fish with starch and milk) showed more similar Henry constant.

Regarding the total influence of temperature on this property, the recipes had their Henry constant presented in different temperatures including 0 and 8°C. There were 8 recipes showing values around 30-45 Pa × ppm^{-1} at 0°C and 35-55 at 8°C. Choosing the recipe (8^{th}) that had the closer composition to the commercial fish cakes used, the Henrys constant was about 32 Pa × ppm^{-1} at 0°C and 38 at 8°C, making an approximation expected of 35 in 4°C, not far from the Henry constant of this work with just a little deviation. Therefore, despite the Henry's

constants found not seeming a reliable in a general look, it turns out to be valid – with little deviation - when compared to another product with very close composition and in the same temperature.

4.1.2 CO_2 final concentration on the package's headspace

In regards to the measured CO2 concentration on the packages at the end of the experiment, the efficiency superiority of the treatment on the hot samples is much larger when compared to the cold ones as can be seen in Figure 4.5.

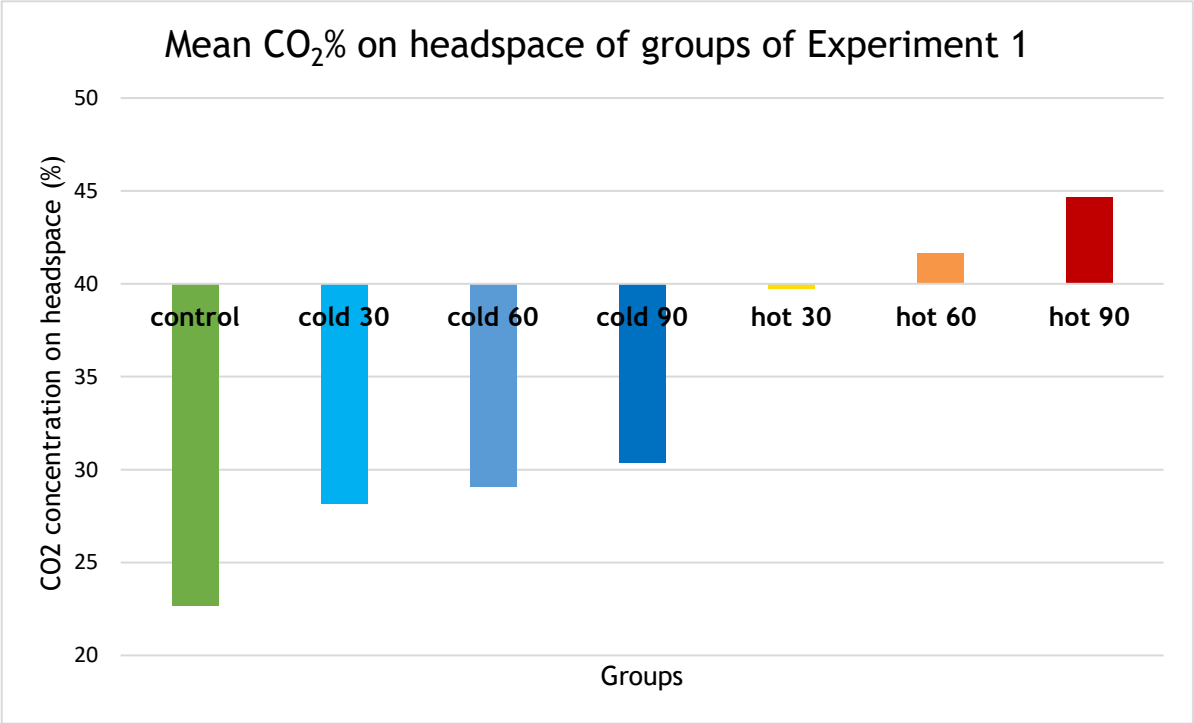

Figure 4.5. Experiment 1 - CO_2 concentration on the headspace of the packages along the storage time, from the view of the initial one (40 %).

In Figure 4.5, highlighting that the samples were packaged in a 40 % MA, the success of the treatment on the hot groups is clear, not only preventing the CO_2 dissolution issue (Hot 30) but even reverting the phenomenon, causing CO_2 desorption from the samples to the headspace. Additionally, it can also be stated that, the technology fits its purpose already in the first half an hour with a small deviation from the initial composition, and granting more 2-3 % of CO_2 to the headspace concentration on the next half hours. On the other hand, although the treatment generates effects on the cold groups, the impact is not so significant. By minute 30 the treatment only dissolved enough CO_2 to cause a 5 % difference in the final MA composition, rising only around 1 % for each extra half hour.

These results, specifically the total superiority in the hot groups, were very unexpected. When discussing the solubility in other studies, all but one mentioned the solubility rate considering the temperature. The studies only address the solubility, which increases with the temperature decrease, causing a misguided general idea that the technology works better on lower

temperatures. This notion was even the one considered when thinking about the predicted results of this work. However, it is hasty to assume some relation between a property of maximum that addresses a system in equilibrium with the rate of absorption. This is not surprising since the only study that covered this point was made 20 years ago. Sivertsvik, Jeksrud, et al. (2004) formulated an equation (3), shown below, relating the absorption rate with temperature. Yet, it was also related to the gas product ratio (in a similar concept of DF) and $\%CO_2$ of the air in contact, this last having the most influence. On top of that, they stated the model did not possess a high correlation (r^2=0.76).

$$\begin{aligned} R_a \times 10^5 \\ = 0.17(g/p - 2.94) + 0.045(T - 276.9) + 0.0132(\%CO_2 - 64.1) \\ - 0.290\left(\frac{(g/p - 2.94)}{0.83}\right)^2 - 0.169\left(\frac{(\%CO_2 - 64.1)}{28.0}\right)^2 \end{aligned} \quad (3)$$

Where R_a is the rate of absorption (s^{-1}), g/p is the ratio of the gas volume and the product volume, T is the temperature (K) and $\%CO_2$ the initial concentration of CO_2 in the atmosphere.

Taking a closer look on the variables definition, it is stated "initial" concentration of CO_2, Hence, the developed equation is designed for CO_2 absorption in, for instance, a MA where the CO_2 concentration decreases through time, and g/p is important to follow this decrease. Hence, the only equation in the literature that may be able to validate this work's findings is actually not appropriate for SGS treatment phenomenon, since the CO_2 in the treatment is in excess, not even decreasing during the treatment time. Still, one piece of information from their work is worth considering, the temperature term coefficient is positive, i.e., although the equation does not provide any additional quantitatively information, it validates the outcome as the higher the temperature, the higher the absorption rate. Therefore, on an equal time frame, the final concentration of CO_2 in a matrix will be as high as higher the temperature is.

An analysis of the linearization of the headspace concentrations to study the effect of temperature and time on the treatment were shown to not be mathematically feasibily to model. Considering packaging the samples in a different MA composition would lead to different relative and absolute changes, shifting the results to smoother or more expressive ones. So the headspace analysis is limited to only comparison between hot and cold groups, not between durations.

4.1.3 Evolution of CO_2 on the samples along the storage time

Concerning the method, it allows us to analyse the process efficacy by the actual initial concentration of CO_2 on the product (before repacking), which is more accurate for efficiency comparison. In addition, this information is independent of the MA packaged. In view of this, the evolution of CO_2 on the matrix is displayed in Figure 4.6 for appreciation and summarized in Table 4.1.

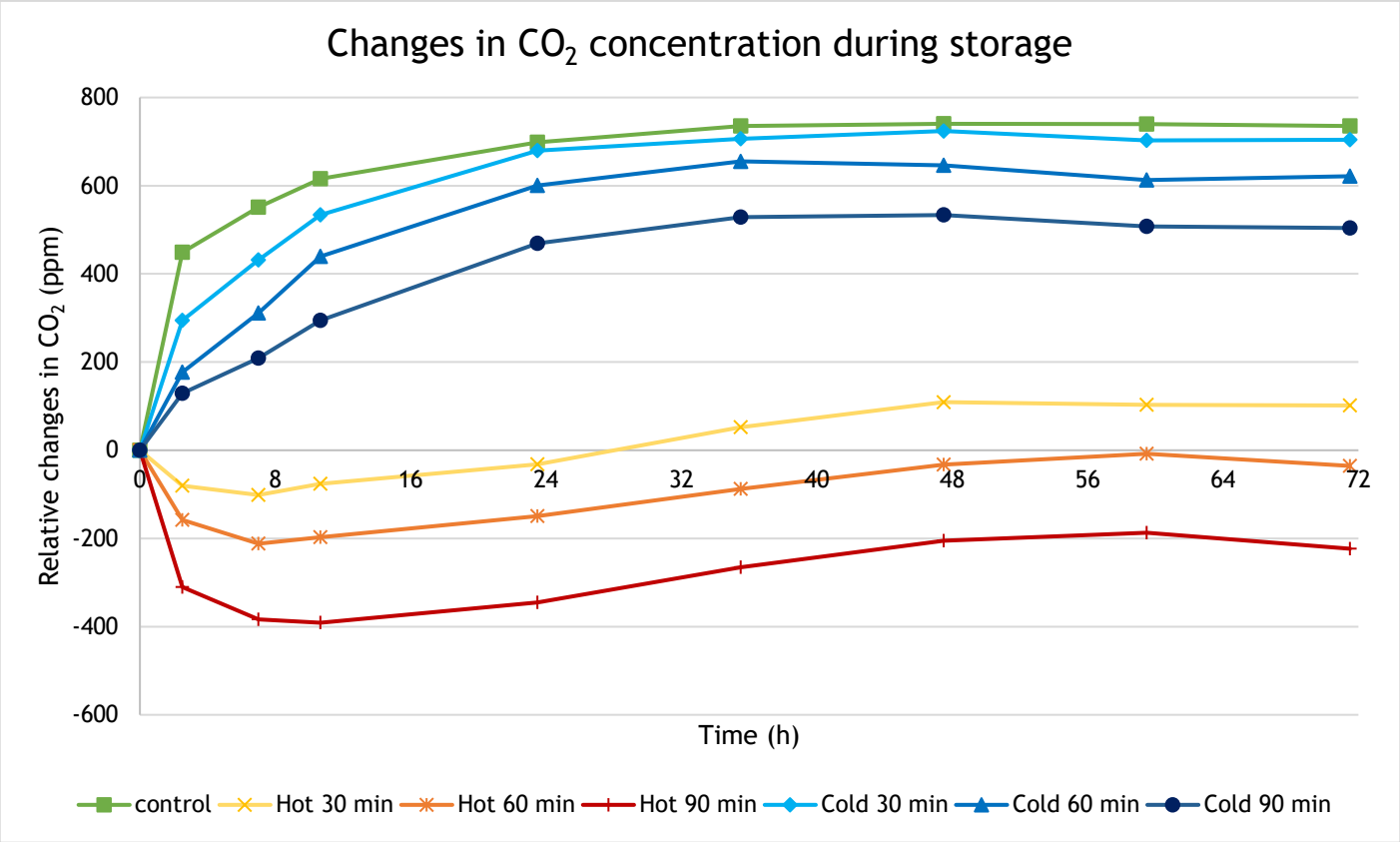

Figure 4.6. Changes in CO_2 concentration in the samples (ppm or mg CO_2/kg product) estimated based on volume change between 2 and 71h of storage in 40 % MA packaging.

At first sight, one main aspect stands out: while the cold group performs as usual increasing the CO_2 curve – as in other studies (Abel et al., 2020; Jakobsen et al., 2022)-, the hot groups do the opposite, intially decreasing the curve. This decrease on the relative change was foreseen from the rise of CO_2 in the headspace of these groups samples, meaning loss of the intial CO_2 in the product as some was transferred to the headspace. The exception to this prediction was the Hot 30 group, which showed a little decrease on the headspace CO_2 concentration - so expecting absortion -, nevertheless its initial behaviour was also desorption. Moreover, the burst in the relative change in the first 2 hours is notable, for positive or negative changes, due to the high distance from the equilibrium in the package.

Table 4.1 – Summary of founds on CO_2 solubility of hot and cold treated fish cakes.

Parameter	Group							P-Value
	Hot 30 min	Hot 60 min	Hot 90 min	Control	Cold 30 min	Cold 60 min	Cold 90 min	
Product CO2 (ppm) initial	1076.5 ± 83.3	1243.9 ± 135.4	1513.5 ± 113.7	-	154.7 ± 52.6	281.6 ± 76.3	411.4 ± 89.6	<0.001
Product CO2 (ppm) equilibrium	1180.6 ± 27.0	1237.1 ± 55.2	1327.7 ± 44.7	709.6 ± 5.7	847.8 ± 85.1	885.4 ± 38.9	912.2 ± 24.6	<0.001
Headspace CO2 (%) equilibrium	39.7 ± 0.9	41.6 ± 1.8	44.7 ± 1.5	22.8 ± 0.2	28.2 ± 2.8	29.2 ± 1.3	30.4 ± 0.8	<0.001
Henry's constant (Pa/ppm)	-	-	-	33.2 ± 1.8	-	-	-	<0.001

The final concentration in the product is simply found by Henry's constant and headspace concentration, while the initial concentration outcomes from the subtraction of the relative difference from the equilibrium (this last one was previously exhibited in Figure 4.6). In accordance with the previously stated, it now becomes even more evident how better the application of the technology is on a hotter system.

The burst of CO_2 dissolution of the hot groups while on significantly higher temperature before the minute 30 also gets clear now, being approximately 7 times bigger than the first half an hour on cold and about 36 % of the maximum possible at 4°C. This could suggest that the temperature has a much higher impact on the solubility than suggested by Sivertsvik, Jeksrud, et al. (2004). On the other hand, considering nature works based on Kelvin and not Celsius, this result seems hard to believe in such little difference in absolute temperature. Even assuming 85°C (358 K) as starting temperature (disregarding the small temperature drop between the heat treatment and the start of the process), and ignoring its drop along the process (as the process happened simultaneously with cooling), the cold samples being at 4°C (277 K) it still seems doubtful. The cold samples were at 277 K, even versus an absurd assumption of constant 358 K, the efficacy difference would be not reliable. Thus, another factor may have influenced such a huge effect on the hot groups.

As mentioned above in the methodology, where the SGS treatment is performed by exposing the samples to close to pure carbon dioxide in excess inside a bag (Rotabakk et al., 2006). it is possible that, besides the bag being of a flexible polymer, the heat transferred from the fish cakes to the bag system (faster than the bag system to outside of it) made the gas to rise its temperature without rising much its volume. The bag is not rigid, however it has a maximum volume. By this token, the possibility of increase of pressure due to rise of the gas temperature while constant volume - which is a fair assumption since we are discussing gas behavior - should be considered. To clarify, the hot fish cakes could have transferred heat inside the bag system (to the carbon dioxide gas) faster than it lost that heat to the outside. Moreover, the heated gas would not be able to expand, limited by the bag's volume, creating a balloon. This would lead to an increase in pressure, while this phenomenon did not happen in the cold groups, causing such a big difference in the outcome.

Beyond that, yet on partially reliable results set, the following 30-minutes periods shows a little linearity increase on the CO_2, but from the minute 30 to 60 there was less CO_2 than from 60 to 90 (167.4 versus 269.6 ppm). This is physically impossible since not only was the temperature lower, but also the mass transfer system was closer to the equilibrium which – by Fick's law – means a lower transfer rate too. Thus, another factor must explain such misleading finding. One reason could be, for instance, the position of the trays in the fridge room: as a room, the temperature is not totally uniform, with the opening of the door making variations and by being big, maybe the difference in the height of the shelves (and the trays were not put on the same level shelves, as this factor was not considered) led to a slower cooling in a group than in the other, resulting in the divergence from theory. Also, from a statistical point of view, considering the quite high standard deviation, these results set start present reasonable findings.

On another note, the cold samples presented a more trustful set of results: Not only the addition from the 30 to the 90 minutes follows Fick's law, as even from time 0 the curve is feasible. Seeing the difference between each half an hour, there is respectively an increase of 154.7, 133.9 and 122.8 ppm. A slow decrease in the dissolution only beside the same conditions, due to the approaching of the system to the equilibrium as more CO_2 is present on the matrix through time.

In this regard, the findings highlight the potential of SGS-technology for an industrial application for heat treated products – and highly preferably – without the need to wait for the product cooling step. The fish cakes exhibited a Henry constant (33.2 ± 1.8 Pa × ppm^{-1} at 4°C) in accordance to other studies regarding specifically its composition (Abel et al., 2018). A similar pattern was observed for the headspace CO_2 concentration, showing a coincidental reduction of headspace CO_2 on cold samples. However, on the hot samples, the treatment was so effective it led to an increase, explained by initial concentrations even higher than the

equilibrium on the hot samples while concentrations closer to 0 than the equilibrium on the cold ones. Another observation is the little impact on the production line schedule, since 30 minutes of treatment is enough to cause considerable effect (36 % of maximum absorption possible). And 30-minute period extensions do not add much more CO_2 (>6 % of maximum) on the still cooling samples and even less in the ones already in the fridge temperature (<5 % of maximum).

Moreover, it even provokes the idea of not accelerating cooling or cooling simultaneously with the treatment, as was done in the method to simulate a time-saving production line. However, it could be worth treating the product first, maybe even keeping its temperature, and only then cooling it. In spite of that, the treatment on cold products showed the practical efficacy SGS as a minor and may have to follow other conditions of application, such as increased pressure, for instance, on the production line, to be worth using.

4.2 Experiment 2: Effect of SGS-technology on quality of MA packaged fish cakes

A second experiment was designed to investigate further the SGS technology's potential to retain the quality attributes of commercial fish cakes. Quality parameters were evaluated for 40 days and on day 72 of refrigerated storage and compared to MA packaged fish cakes without SGS. The final headspace compositions of this experiment's groups are in accordance with the ones of Experiment 1, as shown in Figure 4.7, indicating the experiments are reliable since both replicates showed identical results.

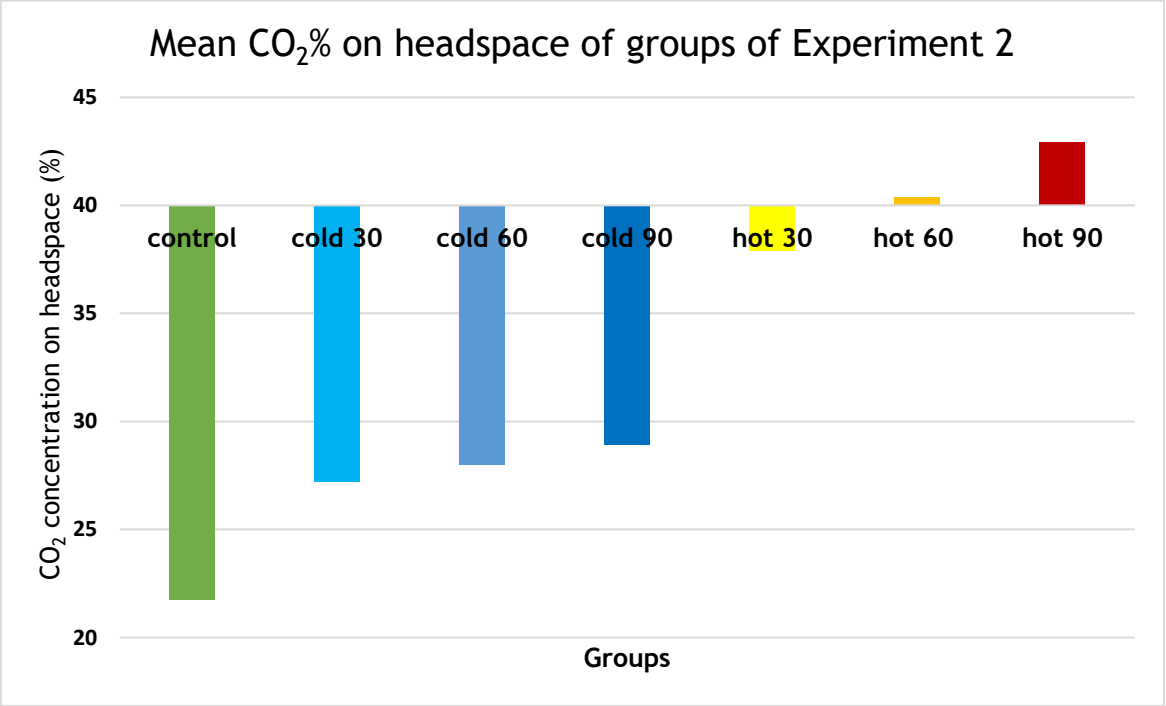

Figure 4.7. Experiment 2 - CO_2 concentration on the headspace of the packages along the storage time, from the view of the initial one (40 %).

4.2.1 Microbiological stability, covered by Experiment 3

In Experiment 2, the initial concentration of APC in the fish cakes was not only below the method's quantification limit of 2.4 log CFU × g^{-1} (Nordic Committee on Food Analysis, 2006), but until the third week, there was no CFU in most of the plates. This low initial contamination level couldn't be directly validated since there is no study addressing microbial growth on fish cakes, not even the one mentioned previously that confirmed the Henry's constant (Abel et al., 2018). Still, it was lower than in studies on the fish that the cakes made of (63 % of the content): saithe *(Pollachius virens*)(Lerfall, Bjørge Thomassen, et al., 2018), white salmon (*Oncorhynchus kisutch*)(Briones et al., 2010), Alaska pollock (*Gadus chalcogrammus*)(Charm et al., 1977).

It was expected to have a lower initial concentration of APC due to the heating process, although during the whole storage of Experiment 2, the APC concentration was still below the minimum for quantification. This can be explained by the heat treatments, calling to mind thermal processes kill bacteria on the product, so extend the shelf life. The second heating of the fish cakes for the experiment - as the suppler already heated it once -, treatment led to so little population, almost esterizing the product. Hence, on the experiment's MA storage conditions, it could not growth to a quantifiable number on the storage time (Experiment 2 count on Appendix A).

Thus, a new experiment without heating the fish cakes from the supplier was designed. Without heating, only the cold groups were left from Experiment 2 for comparison. However, it was known from Experiments 1 and 2 the little difference in headspace concentration, so a small impact was expected on other parameters. This could lead to a difficult distinction of the microbial growth on the Control and cold groups. Hence, a more extended application on cold was necessary.

It was also known that an overnight application was another option producers indicated for including the technology on the line. Therefore, overnight (18h duration) groups were included: a first one with the same MA composition as the other groups (40 % CO_2) and an extra one in a different MA (70 % CO_2). A significant desorption was predicted if samples were packaged in the first MA after such long SGS treatment. Moreover, considering the probable little distinction between the Control and cold groups, the distinction among the cold groups with only half an hour of difference would be even more difficult. Thus, only one of the cold times was elected, since would be redundant to include all groups.

This prediction becomes even more unlikely between cold groups, making the inclusion of all of them very likely to produce redundant results, so one of them was enough. Another major

change was the rise of the DF, since the technology makes available the packaging in higher DFs, it was raised to 1/2 to prove its efficacy in this condition.

Concerning this, 4 groups were set for Experiment 3: Control, 1h-40%, 18h-40% and 18h-70%. And the final headspace concentrations (which are known to be in equilibrium with the CO_2 dissolved in the food) of the final 4 groups of Experiment 3 are displayed below in Figure 4.8.

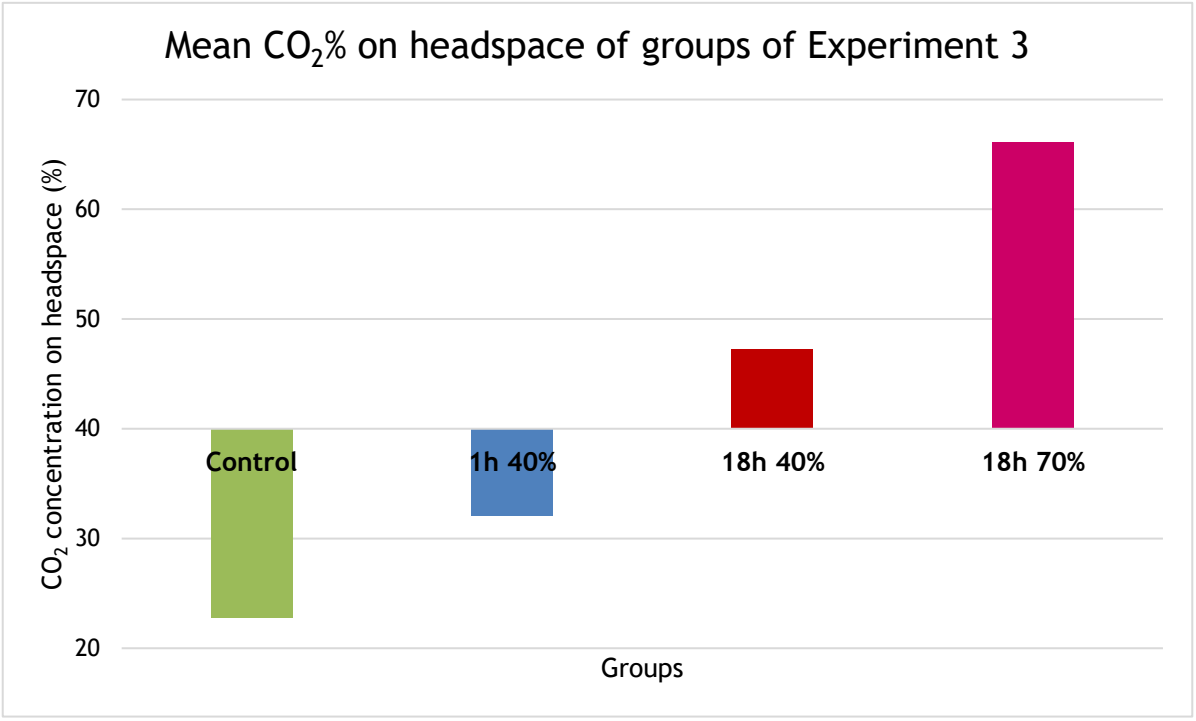

Figure 4.8. Experiment 3 - CO_2 concentration on the headspace of the packages along the storage time, from the view of the initial one (40%).

The Control group achieved a final CO_2 concentration in accordance with the previous Control (around 23 %). The 1h-40% got a little higher value than the cold groups, still close to 30 %, at the same time, the 18h-40% had a number that is close to the Hot 90 group (close to 45 %) and the 18h-70% achieved 66 % - without any signs of collapse as it remained close to the initial concentration.

Once again, the initial concentration of APC in the fish cakes (1.9 log CFU × g^{-1}) was below the method quantification limit of 2.4 (Nordic Committee on Food Analysis, 2006), a value much higher than Experiment 2's and much closer to the initial values for the fish mentioned above - while still below, as it was heated by the supplier before the experiment started. However, once again, in a significant period (15 days) it wasn't detected any statistically distinguished growth. There should be at least some minimal increase, besides the expectation of lag phase (in which there is not so much growth) on the beginning. Thus, considering the limited number of samples, it was decided to skip assessments until close to the Control expected shelf-life time (40 days). Since the objective was to find out the extension on the shelf life of the technology, it could only be equal or longer than the Control. The next sampling point was only

on day 34 (a week before the expected spoilage point) and the following samplings were done weekly, as shown in Figure 4.9. Consequently, the microbial growth tendential line between days 15 and 34 is doted instead of solid to highlight the probable deviation of it from reality (Lerfall, Bjørge Thomassen, et al., 2018).

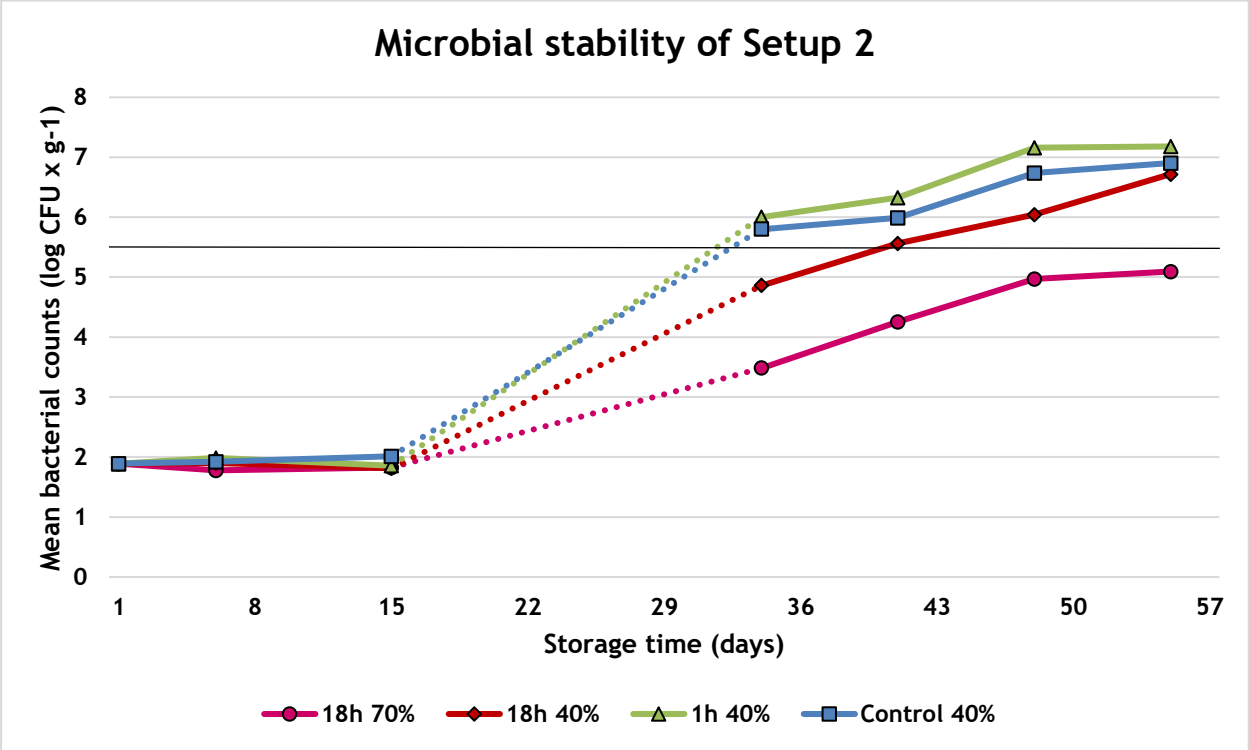

Figure 4.9. Evolution of total aerobic plate counts (APC) of commercial fish cakes pretreated with SGS or air during storage at 4°C by Experiment 3.

There are no specific criteria for spoilage of fish cakes by APC, but several guidelines and studies state APC levels higher than 6 log CFU × g^{-1} as borderline or unsatisfactory quality (Jakobsen et al., 2022). Fish product show logs around 5-6 at the end of their shelf lives (Fogarty et al., 2019) and there is already sensorial rejection of the product at 7 log CFU × g^{-1}(Gram & Huss, 1996). With this in mind, in order to trace a clear shelf-life frontier in this work, as highlighted in the figure above, it was considered 5.5 log CFU × g^{-1} the mark where the product ends its shelf life: closer to the lower bound of the literature range, to highly secure the product's quality until the end of the shelf life to be indicated on this study. In an additional note, shifts in the borderline to the rounded log marks (5 or 6) wouldn't produce much variation on the final shelf-life comparison, however, it would increase the uncertainty: in the Control and 1h-40% groups if lowered and in the 18h-70% if raised. Moreover, H_2S-producing bacteria was never detected.

Following the model of Baranyi and Roberts (1994) to analyze the microbial growth, it is likely that all the samples presented a lag phase of at least 15 days. In addition, it is known that SGS impacts by both extending the lag phase and reducing the growth in exponential phase (Esmaeilian et al., 2021). For this reason, and with the information of the sampling point after

the skip (where the 18h groups had much lower CFU), the lag phase can be predicted of the Control, and the 1h-40% was very close at the end, the 18h-40% may have a little longer lag phase and the 18h-70% a significantly longer one. Graphically, this prediction means the 18h would have lower counts than the dotted, mainly on the begging of the hiatus. Concerning the analysis of the possible maximum specific growth rate(m_{max}), considering the previous predicted extension of the lag-phase by SGS, it is possible to hypothesize a m_{max} above 1 log CFU × g^{-1} per week for the Control and 1h-40% groups and below that value for the 18h ones. Thus, the CO_2 absorbed may not only impact the lag-phase but the exponential-phase too, in accordance to previous studies (Devlieghere & Debevere, 2000).

When sampling restarted at day 34, the Control and 1h-40% were already spoiled with around 6 log CFU × g^{-1}, meaning a shelf life shorter than that, much probably around a month, as the following week's points showed a transition to stationary phase from there. As stated above, from the close final concentration CO_2 between the Control and 1h-40% groups it was already predicted a possible similar behavior among them. On the other hand, the 18h groups seemed to be still in the exponential phase: the 18h-40% was 1 log below, finishing its shelf life a week later and only reaching around the 6 mark two weeks after. The 18h-70% was 2.5 log below the first ones, not reaching the shelf life end until the end of the 55 days of storage, besides being very close to it, less than 0.5 log CFU × g^{-1}.

Ultimately comparing the group's suggested shelf lives, by the adopted high-quality assurance borderline of 5.5 log CFU × g^{-1}, the Control and 1h-40% groups reached about a month of shelf life. A meaningful outcome, the 18h-40% - which supposedly behaves like the Hot 90 group from experiments 1 and 2 - reached around 40 days shelf life, meaning 10 additional days or 1/3 extension. And the most notable outcome: the 18h-70% would surprisingly double the first groups shelf life, only getting spoiled in approximately 60 days. Considering all the factors discussed, the CO_2 pretreatment can be an efficient hurdle to delay microbial growth, similar to previously reported for other fish products packed in MA (Sivertsvik, Jeksrud, et al., 2002; Velu et al., 2013) and more specifically saithe (Lerfall, Bjørge Thomassen, et al., 2018), indicated before as the main component of the fish cakes, meaning a great finding for this industry to consider.

4.2.2 pH

Cocnerning Experiment 2, one of the most likely properties to be impacted by the SGS treatment is the pH. Since the effects of the technology are pointed to be greatly due to pH change by the carbonic formation already explained, in close to a linear relationship between the amount of CO_2 (Sivertsvik, Jeksrud, et al., 2002; Velu et al., 2013). By this token, the samples' pH was measured on each sampling point. It was found that the mean pH of the groups

remaining stable through the storage period, although presenting a clear difference between the hot and cold groups, displayed on Figure 4.10 below.

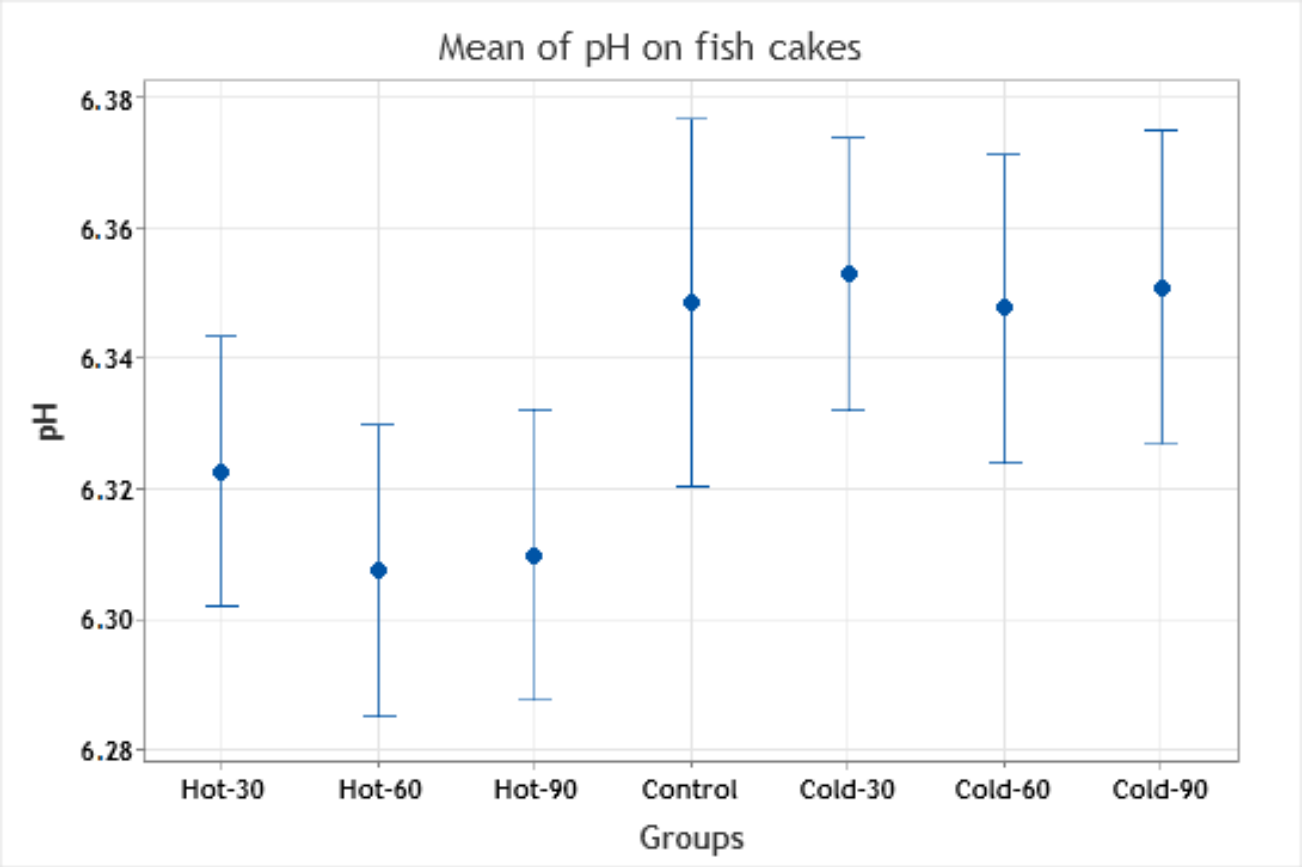

Figure 4.10. Variation on pH of groups.

For the hot groups, there was a little drop in the pH (0.04 units), while there was statistically no drop for the cold groups in comparison to the Control, with a deviation almost as high as the difference between hot and cold groups. The little difference with such high deviation leads to few possible theories relating these results to the previous sections.

First, physically, it appears that pH is not so sensible to CO_2, requiring a bigger gap in CO_2 concentration to express notable differences. Second, the impact on microbial growth of the 18h-40% (group like the Hot 90) was mainly due to CO2 diluted in the food matrix not significantly related to a pH drop. Third, and in opposition to the previous hypothesis, as the 1h-40% (group like the Cold 60) got no effect neither on microbial growth or pH, it may imply that actually bacteria are highly sensitive to pH, and instead, the 18h-40% got such difference due to a little pH drop. Thus, deeper studies on the pH participation concerning the CO2 diluted should be carried out to determine which of these assumptions are valid.

4.2.3 Drip loss

DL from muscle foods occurs due a changed capacity of the muscle structure to retain its natural water (Huff-Lonergan & Lonergan, 2005). As previously stated, two main factors increase drip loss: exposure to high CO_2 concentrations (Sivertsvik, 2007) and, snugging down (Rotabakk et al., 2006). This way, the study of DL in SGS context is ultimately determined by the prevalence of one of those factors. In order to answer this question, the DL of the groups of Experiment 2 are exhibited below on Figure 4.11.

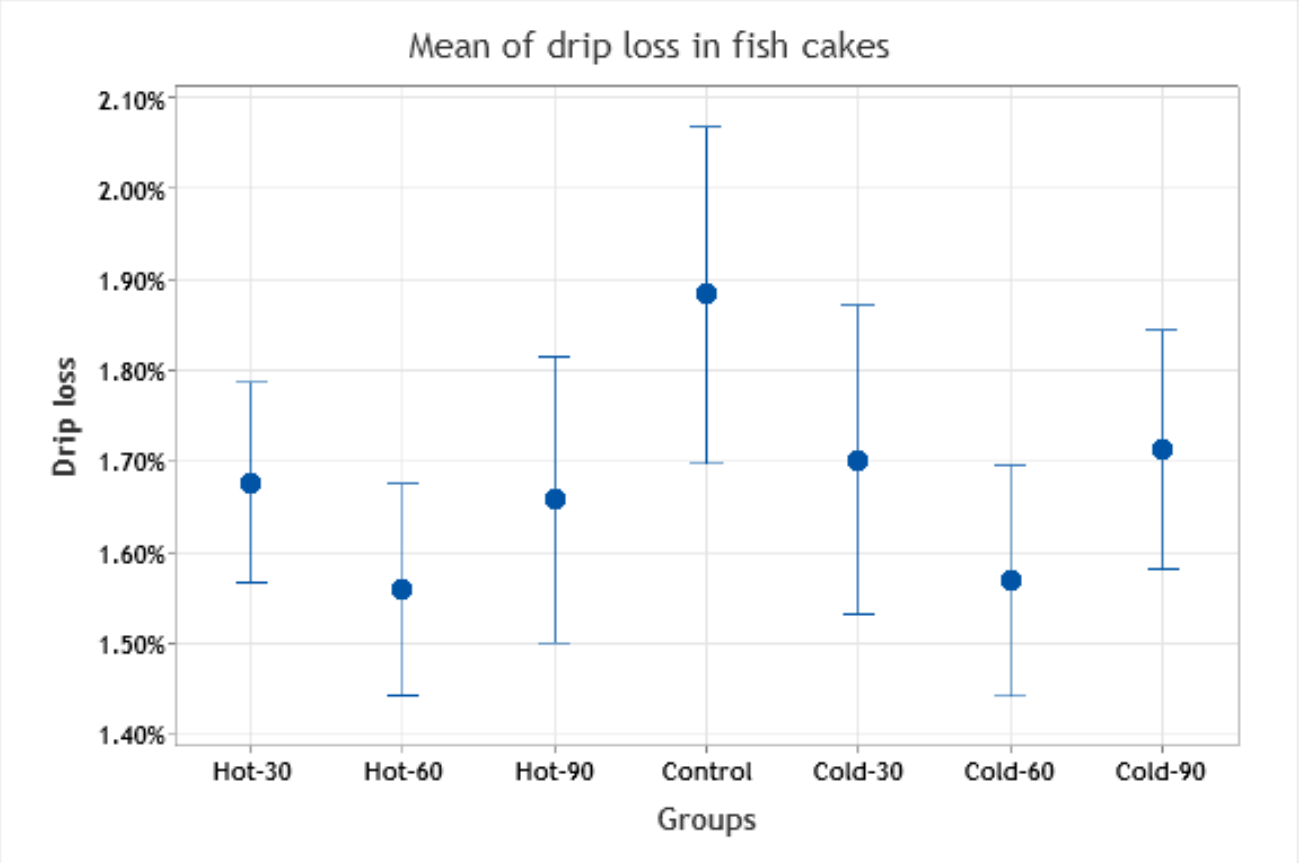

Figure 4.11. Variation on DL of groups.

As expected, the Control group gave the highest DL, due to snuggling of the package and consequential squeezing of the fish cakes. Among the SGS treated groups, they presented similar DL, with the 60 minutes groups having the lowest one, but still statistically the same of the other treated groups. Thus, it was also found that, above a concentration that prevents the snuggling of the package, the concentration of CO_2 or pH didn't seem to impact the DL in the range of the work.

On another note, the evolution of drip loss along the shelf life is also an important quality parameter; therefore, in Figure 4.12, the general tendency of drip loss in the scale of the group variations is shown.

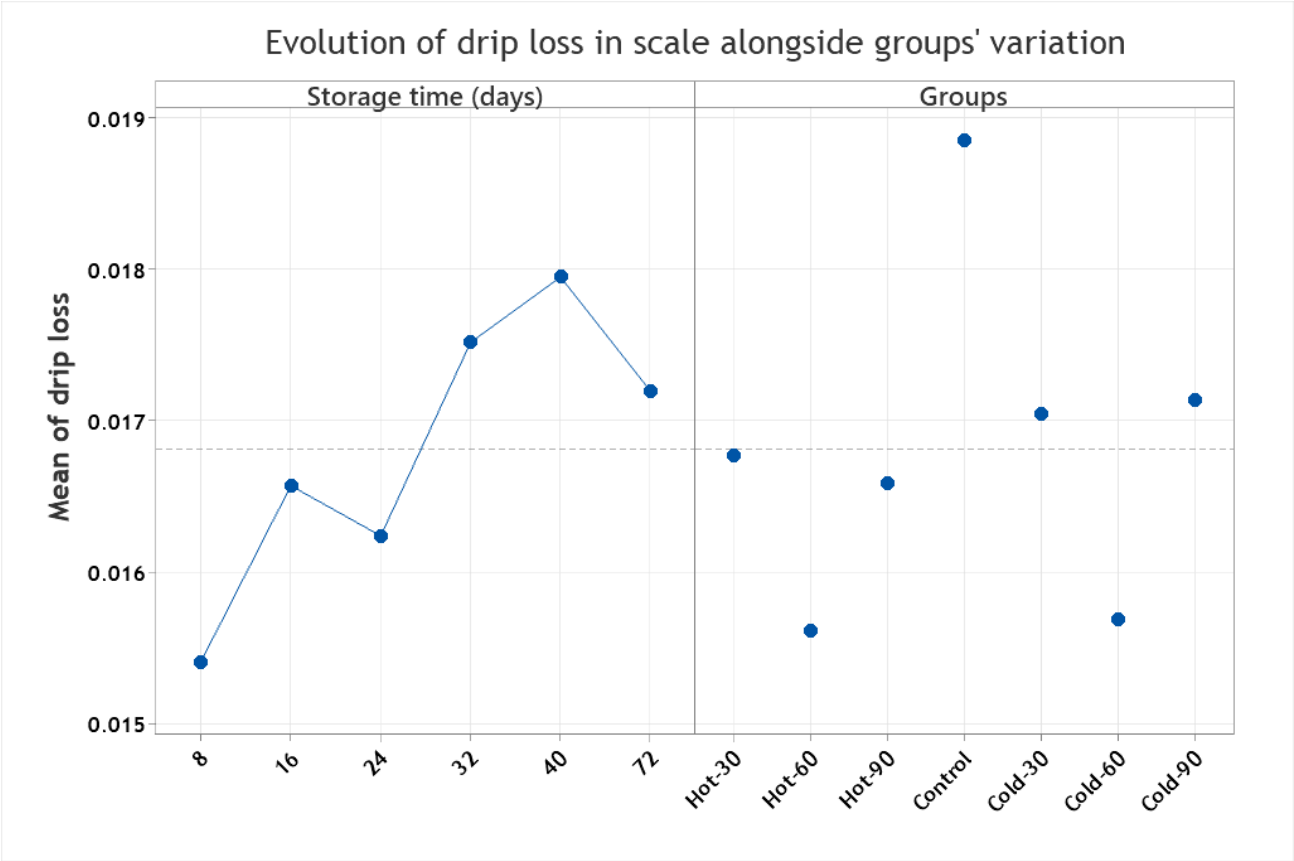

Figure 4.12. Evolution on DL of groups along the storage time with group's variation.

By the evolution of DL, the tendency of the fish cakes to lose more water along the storage time is clear. During all the storage time the product have lost 1,5 % of its weight on the first week and less than 0,5 % of during the rest of the storage. Hence, the practical implication is if this difference could be noticed by consumers. And the texture analysis gives indications on that.

4.2.4 Texture

Texture is a very important aspect for consumers when electing a product to buy. Hyldig and Nielsen (2001) concluded the drier (lower water content) the fish, the firmer it is, beyond the temperature of the product and other factors. Moreover, in the specific case of fish cakes, a crust formed by the heating with a higher resistance than the rest of the product, this crust can get softer or harder. Consequently, this is another sensible textural aspect to be assessed along with firmness; being important to address both aspects as they translate into the product's quality. In light of this, Figure 4.13 shows the effect of SGS treatment in both aspects.

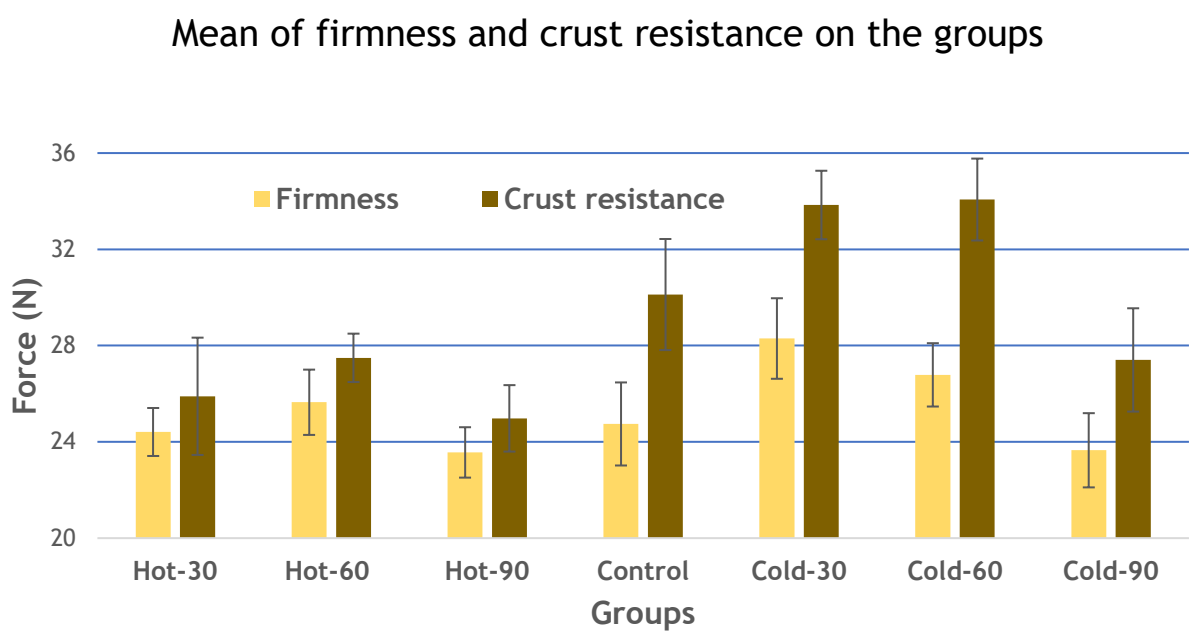

Figure 4.13. Variation on firmness and crust resistance of groups.

Analyzing separately the firmness or the crust resistance of the groups, it seems to have no pattern nor any correlation between them and any other parameter discussed previously. However, if analyzing them together (the difference of force between the texture and crust resistance), it is visible the values have few correspondences to the CO_2 dissolved. The hot groups seem to have a fixed little difference between force from crust resistance to the firmness. On the other hand, the Control and cold groups this difference is quite bigger. Only the Cold 90 group has a difference in between the hot and the cold groups. This way, it's fair to affirm the treatment softens the crust, what only could be defined as a positive or negative effect with a consumer preference study. In another note, the high error is due to the variations on the shape of the fish cakes, being taller and thinner or shorter and thicker.

Still, it is important also to observe the evolution of the sample's texture along time. Since there was an increase in the drip loss through time, it probably has consequences on the texture. Hence, the evolution of both aspects is exhibited in Figure 4.14 below.

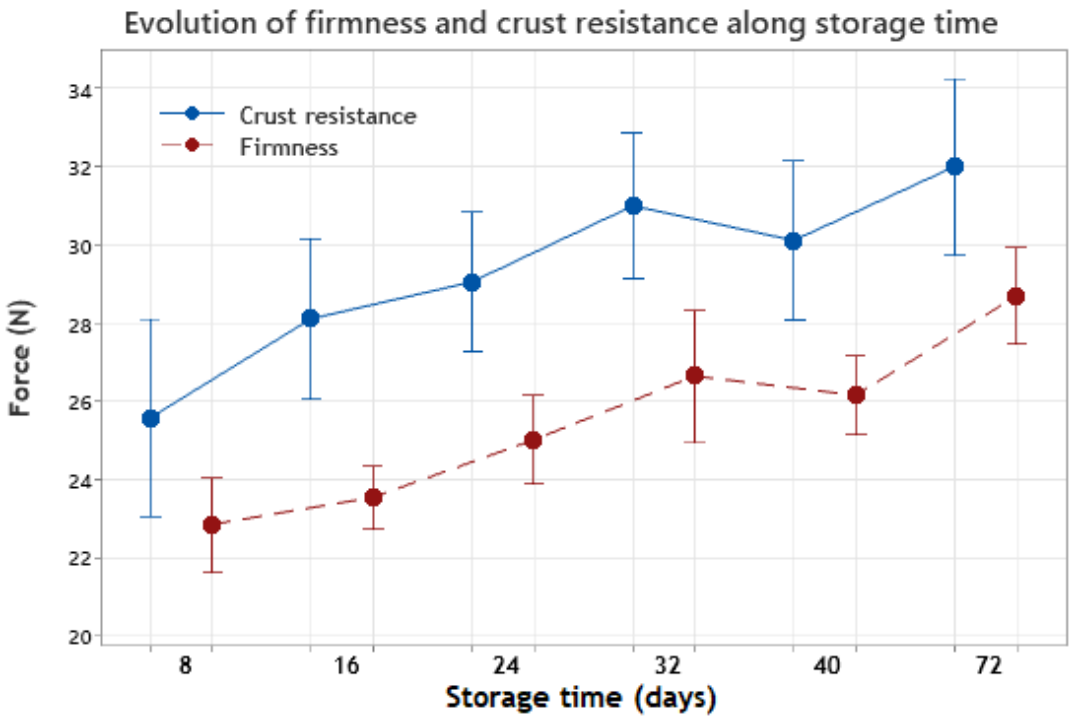

Figure 4.14. Evolution of firmness and crust resistance along the storage time.

From the last figure, it is confirmed the hypothesis related to the drip loss. The fishcake and its crust are getting firmer by time, probably due to drip loss. If crossing this data with the groups ones above discussed, it is fair to say the hot groups presents closer properties of a fresher product along the storage. They presented an opposite tendency to the texture evolution: the product gets stiffer by the time, while the hot groups have a softer crust. In another note, considering the previous figure, it was expected a higher error on the crust resistance than in the firmness, as the groups firmness were much close to each other than the crust resistance.

4.2.5 Quality and shelf-life determination

Taking all the above into account, a general overview on the effect of the SGS process on shelf life and quality can be traced. First, it was not only confirmed the efficacy of the treatment on extending the shelf life of heated fish products in a short time process duration. Accepting the Hot 90 group behaves like the 18h-40%, the treatment provided 10 extra days or 1/3 more shelf life with a 1.5 h treatment. Also, it overperformed, as it doubled the shelf life if applied a longer duration (total of 60 days). This first shelf life is achieved barely impacting the pH of the product end reducing drip loss on some length. However, during this extra shelf-life time, it is possible a reduction on the product's quality, as it is likely to lose drip and get firmer by time. SGS is for sure a technology to be widely adopted in the future, nevertheless, a way to properly introduce it on the production lines must be designed. The actual method is done by batches, what wouldn't fit in a continuous production line. Moreover, other factors, such as pressure, can enhance the time efficiency of it.

5 Conclusion

In this work, soluble gas stabilization, a CO_2 treatment, was applied in heat-treated fish cakes at two different points of the production line (still hot just after the heat treatment or after cooling) and in three different durations (0.5, 1.0 and 1.5 h) to compare the technology time efficiency and efficacy on extending the shelf-life and preserving the quality.

The time efficiency, studied on the gas solubility through a gravimetric method, was shown to be the opposite of the expected as other studies on this technology emphasize the increased solubility of CO_2 by reduction of temperature. However, the treatment on hot achieved 1076.5 ± 83.3 ppm, or 36 % of the maximum solubility in the product by the valid Henry's constant determined, in the first half an hour, while the treatment on cold only achieved 5 %. Moreover, a slightly higher solubility rate was found in the following half hours (>6 %) on the still cooling samples when compared to those already in the equilibrium temperature (<5 %). Hence, this means a high importance of the first half an hour and suggests keeping its temperature for the treatment and only then cooling it.

The effect on shelf-life, indicated by the microbial stability of the product, was not only confirmed as expected from other studies but exceeded the expectations. The group parallel to the cold ones and the Control had a shelf-life of about a month, while the group parallel to the 90-minute-duration hot treatment achieved 10 days more than that, and finally, a more extensively treated group and packaged in higher CO_2 would reach two months of shelf-life if the experiment did not end before. Considering the cost-benefit of the treatment, an economical study would be necessary to evaluate if it is better only to invest 30 minutes for 1/3 extra shelf life or an actual long treatment to double it.

The quality preservation was guaranteed, as the treatment did not negatively affect the quality. In fact, the treatment even enhanced it a little: it had close to no impact on the pH, the drip loss was slightly reduced, and the texture was subtly enhanced. Thus, the CO_2 dissolution possible defections on quality showed by other studies were not confirmed for fish cakes.

Regarding all the factors discussed, it is visible how SGS is a promising technology and very suitable as a synergetic hurdle with thermal processes to answer the challenges of preserving food and mainly fish products. The next step is designing a new method of application of treatment for insertion on a continuous production line since the actual treatment is done by batches, beyond considering other efficiency factors such as pressure. In addition, a chemical analysis of the product spoilage by bacteria should be carried to define better the shelf-life.

6 Assessment of the work done

6.1 Objectives Achieved

The first objective was to examine the solubility of CO2 in fish cakes. The solubility was well characterized with the determination of a valid Henry's constant and results supported by the literature.

The effect on quality parameters was identified and the relation between the treatment and pH, drip loss and texture were discussed.

The shelf-life determination was also successful, with it being enhanced depending on the treatment parameters.

6.2 Other Work Carried Out

An extra experiment (4) was carried out just to confirm the effects, to some length, of packaging food in MA with high concentrations of CO_2 without SGS process. More specifically the snuggling effect of the CO_2 dissolution on the product and the CO_2 on pH. In this light, fish cakes were packaged in MA with progressively higher concentrations of CO_2, From 20 to 100 %, with a 20 % difference between groups and N_2 to balance. Therefore, 5 groups (n=6) were packaged and the results assessed after 72h. The results of this work are available in Appendix B.

6.3 Final Assessment

The work developed for this Master thesis allowed an initial understanding of CO_2 dissolution rate relation with temperature. Also gave indications on the CO_2 impact on shelf-life and quality of fish products. Although it has lots of improvements to be carried, it achieved its goals with the resources available. Specifically, this work suffered with limitation on the initial number of samples twice, with more samples a more precise study could be done.

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Apêndice A - Experiment 2 APC

As stated before, during all storage period of Experiment 2, the APC count stayed below the statistical limit (2.4 log CFU × g^{-1}), as shown on Figure A.1 – Samples with no detected bacterial counts were scored as 1 CFU/g before log-transformation.

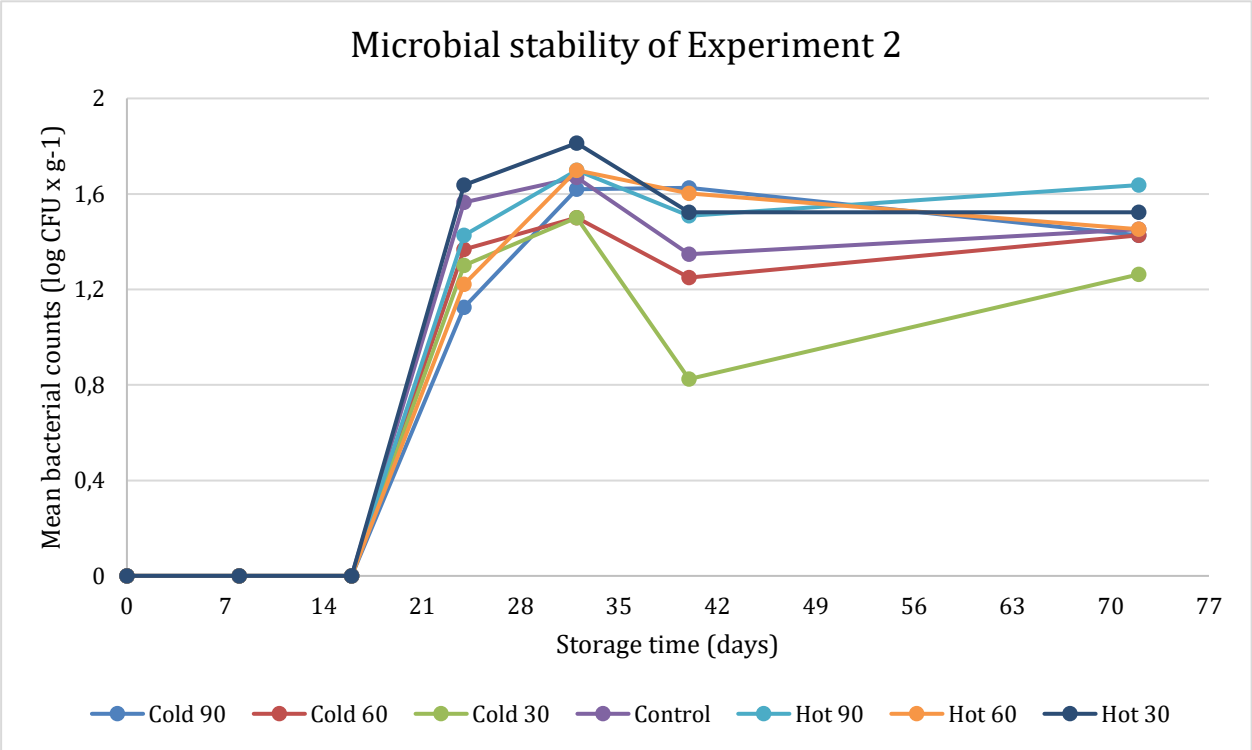

Figure A.1. Microbial stability of Experiment 2

Apêndice B - Extra experiment results

The experiment B produced in resume, three information: the drop of CO_2 concentration in MA without SGS, the visual effect of the phenomenon, and the drop on pH now with a wider range CO_2 in equilibrium.

In this light, first, it's displayed the comparison between CO_2 in the initial MA packaged and final concentration of CO_2 on the headspace on Figure B.1.

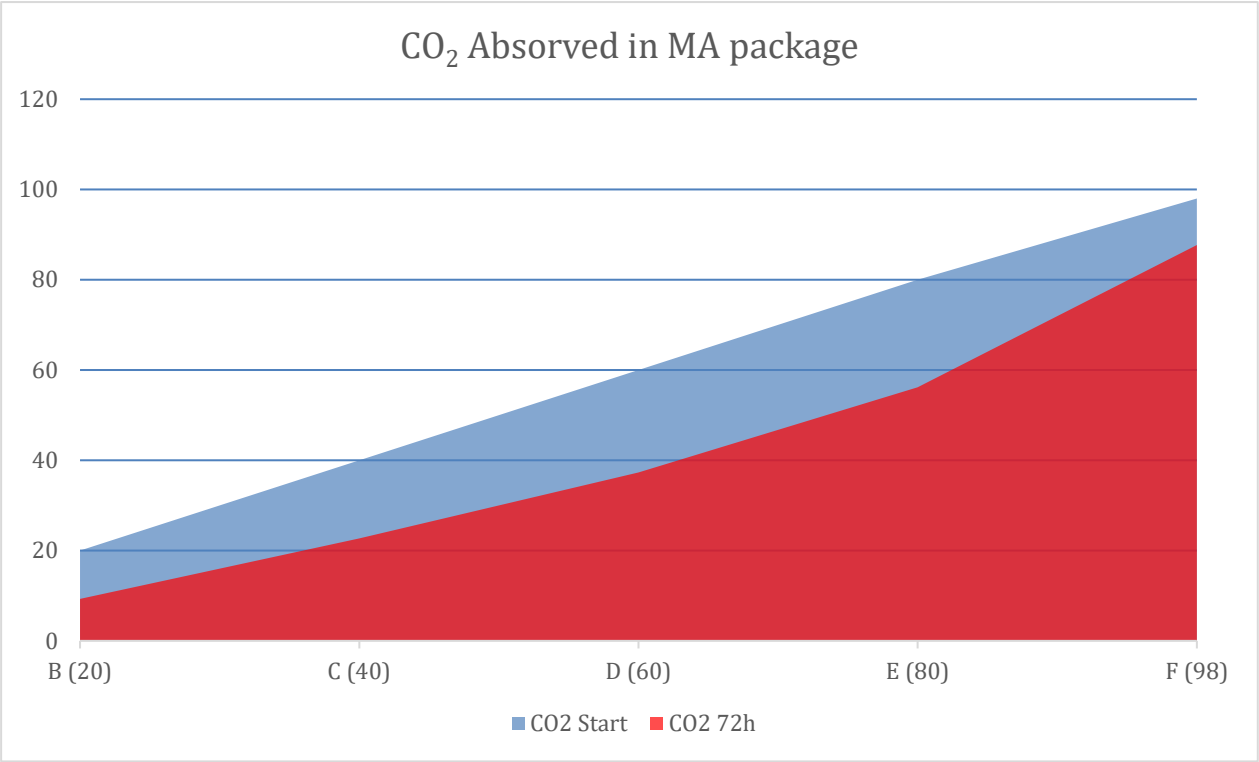

Figure B.1. Difference of headspace CO_2 concentrations of MA packages without SGS.

From the difference between the initial it's visible it grows with the initial CO_2. Excepting the 98% MA, the absolute difference rising slowly from about 11 % to 24 %, but decreasing in relative drop, as there is more total amount of CO_2 in the MA to be absorbed by the same volume of product.

Concerning the pH, it was detected a stronger correlation between CO_2 diluted and pH drop, exhibited on Figure B.2. below.

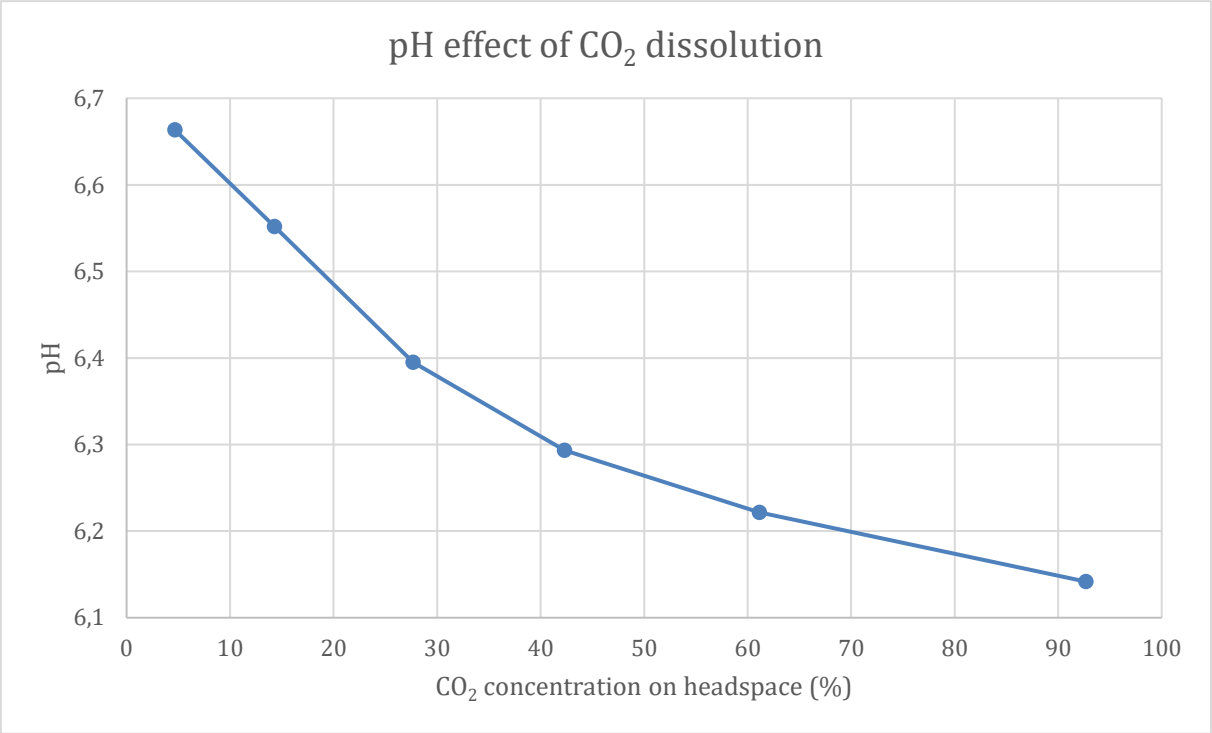

Figure B.2. Correlation between pH and CO_2 equilibrium concentration on headspace

From this data it is possible to see CO_2 has a linear to logarithmic correlation with pH, dropping about 0,1 per 10% in the beginning until about 25 %, then ending dropping 0,33 per 10 % from 60 % and above. What invalidates the findings on the main experiments, since the drop there was much lower, maybe needing a review on this aspect.

Finally, the visual progression of the snugging down effect can be followed, with the picture of 40, 60 and 80% MA from Figure B.3 to B.8.

Figure B.3. Back angle of fish cakes packaged in 40 % CO_2 MA.

Figure B.4. Side angle of fish cakes packaged in 40 % CO_2 MA.

Figure B.5. Back angle of fish cakes packaged in 60 % CO_2 MA.

Figure B.6. Side angle of fish cakes packaged in 60 % CO_2 MA.

Figure B.7. Back angle of fish cakes packaged in 60 % CO_2 MA.

Figure B.8. Side angle of fish cakes packaged in 60 % CO_2 MA.

From these pictures, it becomes visible that packaging up to 40 % does not barely show snuggling effect, what actually realized on the control groups of the main experiments, with the start of this effect, however, from 60% is is unfeasible to directly package in MA without damage.