LWT - Food Science and Technology 201 (2024) 116223

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Impact of soluble gas stabilisation (SGS) technology on the quality of superchilled vacuum-packed salmon portions following different cold chain scenarios

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ARTICLE INFO

Keywords:
Hurdle technology
Cold chain
Soluble gas stabilisation
Superchilling
Salmon portions

ABSTRACT

The soluble gas stabilisation (SGS) technology involves dissolving CO_2 into food at low temperatures before packaging. This study examines the impact of SGS on superchilled vacuum-packed salmon portions under varying cold storage conditions, simulating transport temperatures (*Temperature I*, 0 or 4 °C) and supermarket storage temperatures (*Temperature II*; 0, 4, and 8 °C). The *Prepackaging treatment* (100% CO_2 (SGS) or air (Control)) was conducted in superchilled conditions (−1.6 °C) for 10 h. Despite the temperature shifts, SGS *Prepackaging treatment* significantly resulted in lower aerobic plate counts (APC, $P < 0.001$, $F = 24.5$), psychrotrophic plate counts (PC, $P < 0.001$, $F = 21.1$) and lactic acid bacterial counts (LAB, $P < 0.001$, $F = 19.56$) by extending the microbial lag phases 2.2–3.0 times compared to the controls. The SGS *Prepackaging treatment* and *Temperature II* were the main factors that delayed microbiological proliferation. SGS samples, stored at low temperatures, extended the freshness (K-value) of the salmon portions and were less affected by temperature fluctuations than controls. For the content of biogenic amines, cadaverine concentration was most affected, and *Temperature II* was the main discriminant. Moreover, the fillet colour was minimally impacted by the design variables. However, increased temperatures generally resulted in darker and less reddish fillets. Overall, SGS *Prepackaging treatment* stabilizes the quality of superchilled vacuum-packed salmon portions under various cold chain scenarios.

1. Introduction

The Atlantic salmon (*Salmo salar* L.) is a vital marine food resource and a lucrative export article for Norway. It is particularly renowned for its high-quality and appealing attributes, making it a preferred choice for raw, ready-to-eat products such as sushi and sashimi. The global surge in sushi consumption has propelled Norway to the forefront of salmon exporting ("Norwegian seafood council," 2019). Pre-rigor salmon fillets are processed shortly after slaughtering and are considered value-added due to their rapid market availability and enhanced freshness compared to post-rigor fillets (Skjervold et al., 2001). However, microorganisms are a severe concern in raw and mildly processed salmon (Løvdal et al., 2021; Parlapani, 2021), and therefore, in recent years, extensive research on novel hurdles (e.g. soluble gas stabilisation (SGS) technology (Jakobsen, Gabrielsen, Johnsen, Rotabakk, & Lerfall, 2022), biopreservation (Stupar et al., 2023) purified condensed smoke (Valø, Jakobsen, & Lerfall, 2020) and combination of hurdles (e.g. organic acid combined with dissolved CO_2 (Schirmer et al., 2009) preventing microbial growth, with minimal effect of sensory and nutritional quality have been conducted (Abel, Rotabakk, & Lerfall, 2022; Esmaeilian et al., 2021; Kontominas, Badeka, Kosma, & Nathanailides, 2021).

Furthermore, low-temperature product maintenance is essential in preventing microbiological growth and maintaining salmon quality traits during processing, distribution and storage. Refrigerated fresh seafood is most commonly distributed and stored at temperatures from 0 to 4 °C (Gallart-Jornet, Rustad, Barat, Fito, & Escriche, 2007). However, temperature fluctuations up to 8 °C are commonly observed (Gonçalves & Blaha, 2010, pp. 287–367).

Superchilling is a preservation method that reduces product temperature below its initial freezing point, typically from −1 to −2 °C (Duun & Rustad, 2008), a process reported to increase the shelf life of

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https://doi.org/10.1016/j.lwt.2024.116223
Received 14 February 2024; Received in revised form 23 April 2024; Accepted 16 May 2024
Available online 23 May 2024

muscle food by 1.5–4.0 times relative to traditional chilling (Banerjee & Maheswarappa, 2019). In this temperature range, a fraction of the water freezes in the product; however, enough fluid is still available inside the food to limit freeze damage (Duun & Rustad, 2007). The superchilling process synergistically improves the product shelf-life when combined with vacuum or modified atmospheric packaging (Banerjee & Maheswarappa, 2019). However, it is a significant challenge to maintain an acceptable temperature during the distribution and storage of the superchilled products in the cold value chain (Kaale, Eikevik, Rustad, & Kolsaker, 2011).

Previous research on diverse muscle food products indicates that soluble gas stabilisation (SGS)-combined with thermal and non-thermal methods could effectively control bacterial growth (Abel, Rotabakk, & Lerfall, 2019; Abel, Rotabakk, Rustad, Ahlsen, & Lerfall, 2019; Lerfall, Jakobsen et al., 2018; Mendes & Gonçalves, 2008; Mendes, Pestana, & Gonçalves, 2008; Mendes, Silva, Anacleto, & Cardoso, 2011; Rotabakk, Birkeland, Jeksrud, & Sivertsvik, 2006; Rotabakk, Birkeland, Lekang, & Sivertsvik, 2008; Sivertsvik & Birkeland, 2006). Despite extensive studies on SGS combined with various hurdles, the effect of combining SGS technology and superchilling remains unexplored. CO_2 solubility and temperature are inversely proportional. Hence, chilled CO_2-pre-treated samples exposed to a minor temperature increase due to a

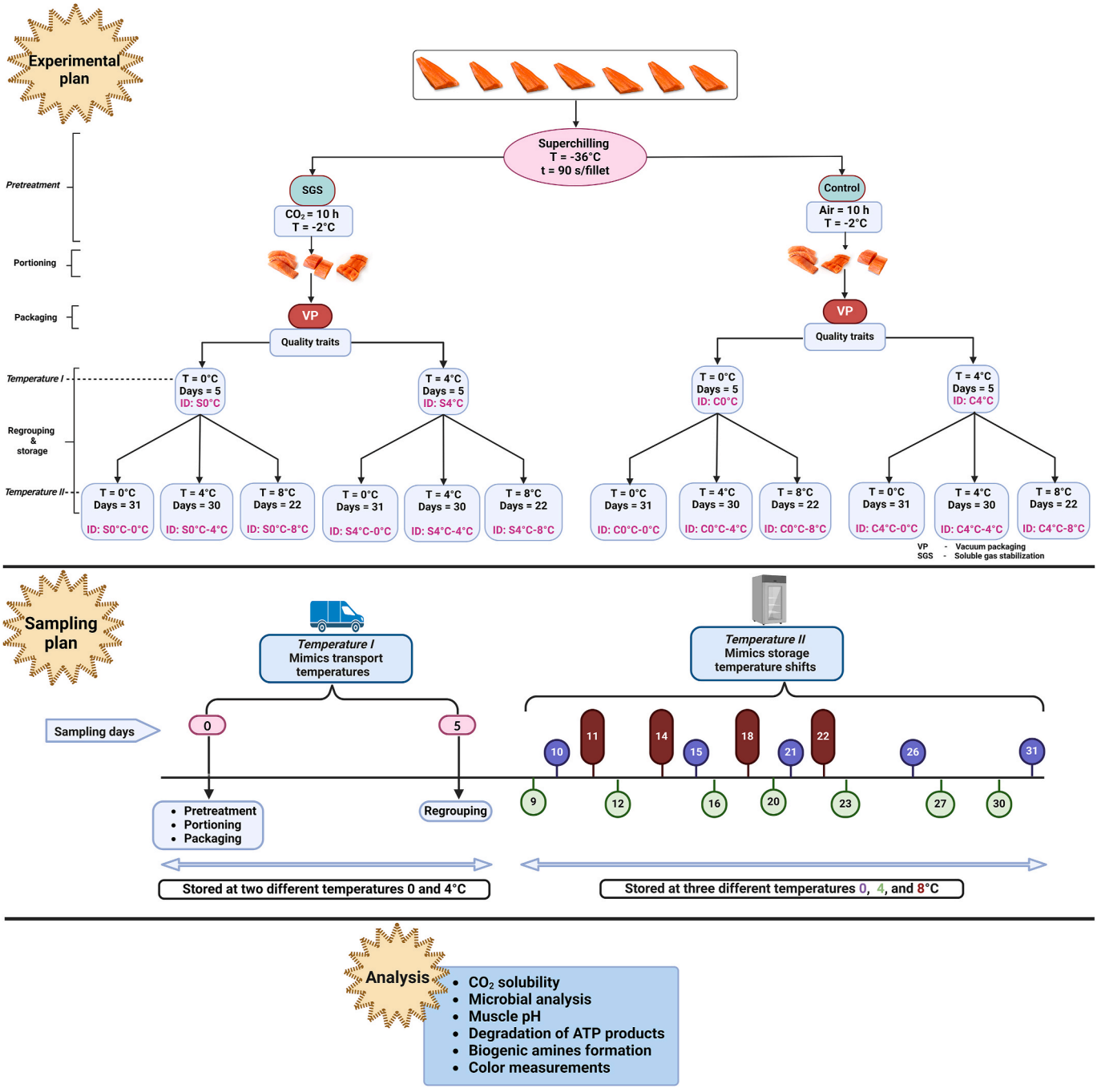

Fig. 1. The experimental design to investigate the impact of *Prepackaging treatment* (SGS or air) and different temperature scenarios (*Temperature I* and *Temperature II*) in the cold chain, on the quality of pre-rigor filleted superchilled and vacuum-packed salmon portions. *Temperature I* (0 or 4 °C) mimics two possible transportation temperatures, and *Temperature II* (0, 4 and 8 °C) simulates different storage conditions in the retail segment. Microbiological analysis was regularly performed as described in the sampling plan, while chemical and colourimetric analysis was performed on days 0, 5, and 11. The image was created with BioRender.com.

broken cold chain may cause adverse effects on food products by releasing CO_2 (Sivertsvik, Jeksrud, & Rosnes, 2002). Thus, this study aims to examine the impact of SGS on the quality of superchilled vacuum-packed pre-rigor salmon portions under varying cold storage conditions, simulating transport and supermarket storage scenarios.

2. Materials and methods

2.1. Raw material

Pre-rigor Atlantic salmon (*Salmo salar* L.) fillets (n = 14) were purchased from SalMar, Norway, and transported on ice in polystyrene (EPS) boxes to NTNU's laboratory within 5 h after slaughtering. At arrival, the salmon muscle's pH was 6.81 ± 0.11 (n = 3), measured in random fillets using a Testo 206 pH instrument (Testo SE & Co., Germany).

2.2. Experimental design

The experiment was designed to study the impact of SGS *Prepackaging treatment* and different temperature scenarios (*Temperature I* and *Temperature II*) in the cold chain on the overall quality of pre-rigor filleted superchilled and vacuum-packed salmon portions (Fig. 1 was modified from (Gurusamy, 2023)).

The salmon fillets were superchilled using an impingement freezer (Frigoscandia, model: Laboratory, FMC FoodTech) at an air temperature of −36 °C with a retention time of 90 s. After 1 h of resting at refrigerated conditions (in EPS-boxes), the salmon fillets were divided into two groups and treated in air only (control) or conducted to an SGS treatment using 100% CO_2 (SGS). The air and SGS treatments were conducted in sealed high-barrier PA/PE bags (Maske AS, Trondheim Norway) filled with either air (Control) or 100% CO_2 (SGS) (Jakobsen et al., 2022) and stored at superchilled conditions (−1.6 °C) for 10 h.

The fillets were then portioned and vacuum-packaged using a Webomatic SuperMax s3000 machine (Webomatic, Germany), which gave rise to two categories: (i) superchilled SGS-treated vacuum-packed samples and (ii) superchilled vacuum-packed samples (Controls). Both categories were re-grouped and stored at 0 and 4 °C for 5 d (*Temperature I*). *Temperature I* mimic two possible transportation temperatures from the producer to the retail storage. On the 5th day, the samples were again re-grouped and stored at three different temperatures (*Temperature II*; 0, 4, and 8 °C) throughout the storage experiment (up to 31 d). *Temperature II* simulates different storage conditions in the retail segment. The experimental design resulted in a total of twelve experimental groups.

Separate samples were used for microbiological analysis of 10 ± 2 g to prevent contamination. Samples in the weight range of 70–150 g per portion were taken for chemical analysis and colour measurements. Microbial parameter sampling was conducted regularly, as described in Fig. 1, and chemical analysis and colour measurements were carried out on days 0, 5, and 11. Samples to be analysed for degradation products of ATP and biogenic amines were immediately frozen at −80 °C until analysis. In addition, the amount of dissolved CO_2 was measured using the volumetric method described by Rotabakk, Lekang, and Sivertsvik (2007) and later modified by Abel, Rotabakk, Rustad, and Lerfall (2018) (see chapter 2.6).

2.3. Microbial parameters

Random samples (n = 3 per group per sampling) from each group were analysed at each sampling point. Around 10 ± 3 g of fish muscle was diluted with 90 mL of peptone water containing 0.1 g of peptone and 0.85 g of NaCl in 100 ml of deionized water. Then, the homogenisation was carried out for 1 min using a stomacher machine (IUL Masticatar, Spain). Serial dilutions were carried out with peptone water (NMKL., 2010). Psychrotrophic aerobic bacteria and heat-sensitive bacteria were detected using the Long and Hammer agar (LHA), and the plates were incubated at 15 ± 1 °C for 6 d (NMKL., 2006). The Lactic acid bacteria were enumerated in de Man, Rogosa, and Sharp agar (MRS) (CM0361, Oxoid Ltd.), incubated anaerobically at 25.0 ± 1.0 °C for 5 d (NMKL., 2007).

2.4. Chemical parameters

2.4.1. Extraction protocol

Frozen fish samples (3 g, n = 3 per group per sampling) were grated using a kitchen grater and mixed up with trichloroacetic acid (TCA, 6 g/100 mL, 15 mL) using an IKA-T25 digital ULTRA TURRAX (IKA-Werke GmbH & Co. KG, Germany). Then, the homogenised samples were centrifugated (5000 rpm, 4 °C, 10 min) using a Kubota-1700 centrifuge (Kubota Corporation, Tokyo, Japan), before the supernatant was transferred into new tubes and further used for the quantification of ATP degradation products and biogenic amines. All extracts were stored at −80 °C until further analysis.

2.4.2. ATP degradation products, K- and H-values

An aliquot (~5 mL) of the extract was adjusted to a pH of 6.25 using KOH (1 M), then centrifuged (12,000 rpm, 4 °C, 10 min) using a Rotina 420 R centrifuge (Andreas Hettich GmbH & Co. KG, Germany) before filtered to a final volume of approximately 1 mL using a nylon filterer (0.25 μm). After filtration, the samples were transferred to HPLC vials (Agilent, part. Nr. 5182-0716) and stored at −80 °C until further analysis.

ATP degradation products were separated chromatographically using a Poroshell 120 porous column (ECC18 3.0 × 100 mm, with porous size 2.7 μm) protected with a Poroshell 120 Fast Guard (3.0 × 5 mm, Sub-2 μm) connected to an Agilent 1260 HPLC system (Agilent Technologies, Paolo Alto, CA, USA) connected to an Agilent 1260 diode array UV–Vis detector after a modified method by (Sellevold, Jynge, & Aarstad, 1986). The ATP degradation products were detected at 210 nm (Adenosine triphosphate (ATP) and adenosine diphosphate (ADP)), and 260 nm (adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx)). The products were eluted isocratic with a ramping flow rate, as described by Lerfall, Thomassen, & Jakobsen (2018). The mobile phase consisted of potassium hydrogen phosphate (KH_2PO_4, 0.215 mol/L) and tetrabutylammonium hydrogen sulphate (0.0023 mol/L) diluted in acetonitrile (C_2H_3N, 3.5 % in water). The mobile phase pH value was adjusted to 6.25 using 0.5 mol/L KOH. All ATP degradation products were then quantified as described by Lerfall, Thomassen, & Jakobsen (2018).

The K-value and H-value were calculated by using the following equations (Hong, Regenstein, & Luo, 2017):

$$\text{K-value (\%)} = [(\text{HxR} + \text{Hx}) / (\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (1)$$

$$\text{H-value (\%)} = [\text{Hx} / (\text{IMP} + \text{HxR} + \text{Hx})] \times 100 \quad (2)$$

2.4.3. Biogenic amines

An aliquot (2 mL) of the extracts prepared (as described in section 2.4.1) was added with benzoyl chloride (2% w/v, 1 mL), made up with acetonitrile (99% Sigma-Aldrich, CAS: 98-88-4)) and vortexed for 1 min. The mixture was rested at room temperature (20 min) before adding saturated NaCl (2 mL) and vortexed for 10 s. Biogenic amines were then extracted by adding diethyl ether (two times with 2 mL). The upper organic layer was then transferred to a clean tube and evaporated to dryness (N_2, 30 °C) before the residue was dissolved in a mixture of acetonitrile and water (90:10) and filtrated using a nylon filterer (0.25 μm).

Biogenic amines were quantified using the method described by Lerfall, Thomassen, & Jakobsen (2018). The benzyl amines were

separated chromatographically using a YMC-Triart PFP (100 × 2 mm, 1.9 μm) UHPLC column linked to an Agilent 1290 chromatography (Agilent Technologies, Paolo Alto, CA, USA) and an Agilent 1260 diode array UV–Vis detector. The loaded samples were detected at 254 nm using H_2O/acetonitrile-based elution.

2.5. Colorimetric analysis

A digital colour imaging system (DigiEye, VeriVide Ltd., Leicester, UK) was used for the colour analysis. Fish samples were kept in a standard lightbox (6400 k), the test area in the fish samples was manually selected, and images were taken by a digital camera (Nikon D7000, 35 mm lens, Nikon Corp., Japan). The images were then examined using DigiPix version 2.8.0.2 (VeriVide Ltd. Leicester, UK), and the output was given as CIE values (L^*, a^*, and b^*) (CIE, 1987).

2.6. Calculation of CO_2 solubility

To calculate the efficiency of the SGS *Prepackaging treatment* in terms of dissolved CO_2, 16 salmon portions (80 ± 2 g per sample) pretreated with either air (n = 8) or SGS (n = 8) were re-packaged into 230 mL semi-rigid crystalline polyethylene terephthalate (CPET) trays (C2125-1 A, Færch Plast, Holstebro, Denmark) were used. The packaging system used was a semi-automatic tray-sealing packaging machine (TL250, Webomatic, Bochum, Germany). When the final vacuum pressure of 2500 Pa was obtained, the samples were flushed with the pre-set MA gas mixture (60% CO_2 balanced with N_2) prior to application of a cover film comprised of a 40 μm combination of polyethylene (PE), ethylene vinyl alcohol (EVOH), polyamide (PA), and polyethylene terephthalate (PET) (Topaz B-440 AF, Plastopil, Almere, The Netherlands). The oxygen transmission rates (OTR) were 66–78 $cm^3 \times 25\ \mu m\ x\ m^{-2} \times 24\ h^1\ x\ bar^1$ at 23 °C for the tray and 2.5 $cm^3 \times 40\ \mu m\ x\ m^{-2} \times 24\ h^1\ x\ atm^1$ at 23 °C for the cover film. The packaging protocol resulted in a sample filling degree of approximately 1:3. The product density was determined to be 1080 kg/m^3 (Abel, Rotabakk, & Lerfall, 2020), and the trays volumetric changes were followed as a function of 0, 1.5, 12.5, 17.5, 25, 37.5, 48.5, 62, 72.5, 108.5, 132.5, and 156.5 h refrigerated storage (4 °C). Moreover, the headspace gas composition at the final sampling was measured using the Checkmate 9900 PBI CO_2 and O_2 sensor (Dansensor, Ringsted, Denmark). The amount of absorbed CO_2 was then calculated by using equation (3).

$$C_{CO_2}^{t=\infty} = \frac{1000\ x\ P\left(v_g^{t=0} - v_g^{t=\infty}\right)\ x\ MwCO_2}{R\ x\ T\ x\ w_f} \quad (3)$$

where,

$C_{CO_2}^{t=\infty}$ = the total absorbed CO_2 (ppm) by the product

P = absolute pressure (Pa)

V_g = gas volume (m^3) at the initial and equilibrium stage.

$MwCO_2$ = molecular weight of CO_2

R = gas constant.

T = absolute temperature (K)

W_f = weight of the product (kg)

The volumetric change is ascribed solely to the amount of dissolved CO_2; hence, the consumption or generation of gas due to microbial activities and change in partial pressure of oxygen and nitrogen are disregarded (Sivertsvik et al., 2002). Henry's law (equation (4)) states that once a sample reaches equilibrium with the surrounding gas, the amount of CO_2 in the headspace is relative to the amount of absorbed CO_2 in the sample (Schumpe, Quicker, & Deckwer, 1982):

$$P_{CO_2}^{t=\infty} H_{CO2,p}\ x\ C_{CO_2}^{t=\infty} \quad (4)$$

where,

$P_{CO_2}^{t=\infty}$ = the partial equilibrium pressure of CO_2 in the headspace gas (Pa)

$HCO_{2;p}$ = the temperature-dependent Henry's constant for CO_2 in the product (Pa/ppm)

Henry's law was used to calculate the amount of CO_2 dissolved during the SGS *Prepackaging treatment*. The amount of dissolved CO_2 will correspond to the amount of CO_2 dissolved in the product at the point of vacuum packaging further investigated in the storage experiment described in section 2.2.

2.7. Microbial growth prediction and statistical analysis

ComBase (www.combase.cc) was used to predict microbial growth. The tool uses average log values of the bacterial count and incubation time to predict the growth curves. The data were tested by fitting them into the primary model of (Baranyi & Roberts, 1994). The statistical analysis of the data was evaluated using IBM SPSS statistical software 27. The microbial data was presented as log-transformed data (bacterial count), and other data are represented as mean ± standard error. The alpha level was set as 5%. A general linear model (GLM) was used to predict the main effects by using design variables *Prepackaging treatments* (SGS or air) distribution and storage temperatures (*Temperature I* and *Temperature II*) as fixed factors. One-way ANOVA was preferred to analyse the comparison between and among the experimental groups throughout the storage days. Furthermore, one-way ANOVA and Tukey's post hoc test were selected to evaluate the experimental groups' differences. Besides, a Person's correlation analysis was used to correlate the linear relationship between the variables.

3. Results and discussion

In this study, the impact of *Prepackaging treatment* and different temperature distribution scenarios (*Temperature I* and *Temperature II*) on the quality of superchilled vacuum-packed salmon portions were followed through a storage trial (Fig. 1). The design variables, *Temperature I* (0 or 4 °C), mimic two possible transportation temperatures, while *Temperature II* (0, 4 and 8 °C) simulate different storage conditions in the retail segment.

3.1. Dissolved CO_2 and muscle pH

The SGS *Prepackaging treatment* was conducted at −1.6 °C for 10 h, resulting in a CO_2 concentration of 475 ± 142 ppm corresponding to the amount of CO_2 enclosed in vacuum-packaged samples pretreated with SGS. Generally, lowering the treatment temperature towards the initial freezing point (for salmon −1.1 °C (Kaale & Eikevik, 2016)) will increase the CO_2 solubility (Gil, 2011). However, the amount of dissolved CO_2 in superchilled Atlantic salmon was approximately 1/3 compared to previously reported levels in SGS pretreated samples (Abel et al., 2018; Abel et al., 2020; Jakobsen et al., 2022). The solubility of CO_2 in muscle foods is affected by various factors, including the proximate composition and temperature (Abel et al., 2018). The large difference in observed solubility between the present study and cited literature cannot be solely explained by the difference in processing time (10 h vs 16–18 h). So likely, other factors, such as, e.g., the formation of small ice crystals under superchilled conditions, affect the amount of water available for dissolving CO_2 (Kaale et al., 2011). Another factor that might influence this is that the experimental protocol measuring the CO_2 solubility was conducted at 4 °C, although the SGS treatment was performed under superchilled conditions (−1.6 °C). Increasing the temperature after repackaging, in MA trays, will theoretically release CO_2 from the product, disturbing the product-headspace CO_2 equilibrium and thus affecting the Henry's constant. In the present study, Henry's constant was estimated to be 47.2 ± 6.3 Pa/ppm, approximately 4–10 Pa/ppm higher than previously reported by Abel et al. (2020); Jakobsen et al. (2022). Hence, an underestimation of dissolved CO_2 during the SGS *Pretreatment packaging* is likely.

The experimental design significantly affected the muscle pH, giving,

on average, a lower pH of samples treated with CO_2 before vacuum packaging. The main discriminant was found to be the *Prepackaging treatment* ($P < 0.001$, $F = 47.6$), followed by the design variable *Temperature II* ($P < 0.001$, $F = 11.3$). Moreover, the design variable *Temperature I* was insignificant ($P > 0.107$), indicating the observed pH difference at day 5 to be solely caused by the CO_2 dissolved during the SGS *Prepackaging treatment*. The lower pH of SGS treated compared to control samples (Day 5: 6.13 ± 0.06 versus 6.26 ± 0.08, respectively) is in the range previously observed in similar studies (Esmaeilian et al., 2021; Jakobsen et al., 2022) as dissolved CO_2 reacts with water in the salmon portions, producing carbonic acid (Sivertsvik et al., 2002).

3.2. Effects of prepackaging treatment, distribution and storage temperatures on microbial quality and growth kinetic

Regardless of temperature scenarios, the SGS *Prepackaging treatment* significantly resulted in lower counts of APC ($P < 0.001$, $F = 24.5$), PC ($P < 0.001$, $F = 21.1$) and LAB ($P < 0.001$, $F = 19.56$) during storage (Fig. 2). The main effect of the design variable *Temperature I* was insignificant ($P_{APC} > 0.057$, $P_{PC} > 0.38$, $P_{LAB} > 0.983$), while *Temperature II* was the design variable of highest importance for microbial development ($P_{APC} < 0.001$, $F = 61.9$; $P_{PC} < 0.001$, $F = 42.03$; $P_{LAB} < 0.001$, $F = 46.5$).

During the first five days, the samples were subjected to either 0 or 4 °C (*Temperature I)*. Combining SGS pretreatment and 0 °C (S0°C group) resulted in an APC level of 2.80 ± 0.01 log CFU/g, significantly lower than for the other groups (One-Way-ANOVA, $p = 0.002$). The APC range of the other groups was 3.1–3.3 log CFU/g. The same pattern was not observed for PC, where the C4°C group had significantly lower counts (2.50 ± 0.01 CFU/g) than the other groups (One-Way-ANOVA, $p = 0.026$). LAB counts were significantly lower for the SGS groups (S0°C and S4°C) (One-Way-ANOVA, $p = 0.003$). On day 5, the samples were transferred to three different storage temperatures (0, 4, and 8 °C; *Temperature II*). The evolution of APC, PC, and LAB in the different samples at group levels is given in Fig. 2.

The SGS *Prepackaging treatment* significantly prolonged the lag phase of APC, PC, and LAB by approximately 2.2–3.0 times compared to the control groups at all temperature scenarios tested (Table 1). Combining SGS *Prepackaging treatment* with a constant storage temperature of 0 °C (*Temperature I* and *II*) most successfully prolonged the lag phase of APC to 9.8 ± 0.2 and 7.5 ± 1.0 d for S0°C-0°C and S4°C-4°C groups compared to 2.4 ± 3.4 and 2.7 ± 1.6 d for the control groups C0°C-0°C and C4°C-4°C, respectively (Fig. 2a–Table 1). A temperature switch from 0 to 4 °C or 4 to 0 °C between *Temperature I* and *Temperature II* also prolonged the APC lag phase (in the range of 4.8 ± 1.7–5.9 ± 2.8 d) compared to the control samples (Fig. 2b–Table 1).

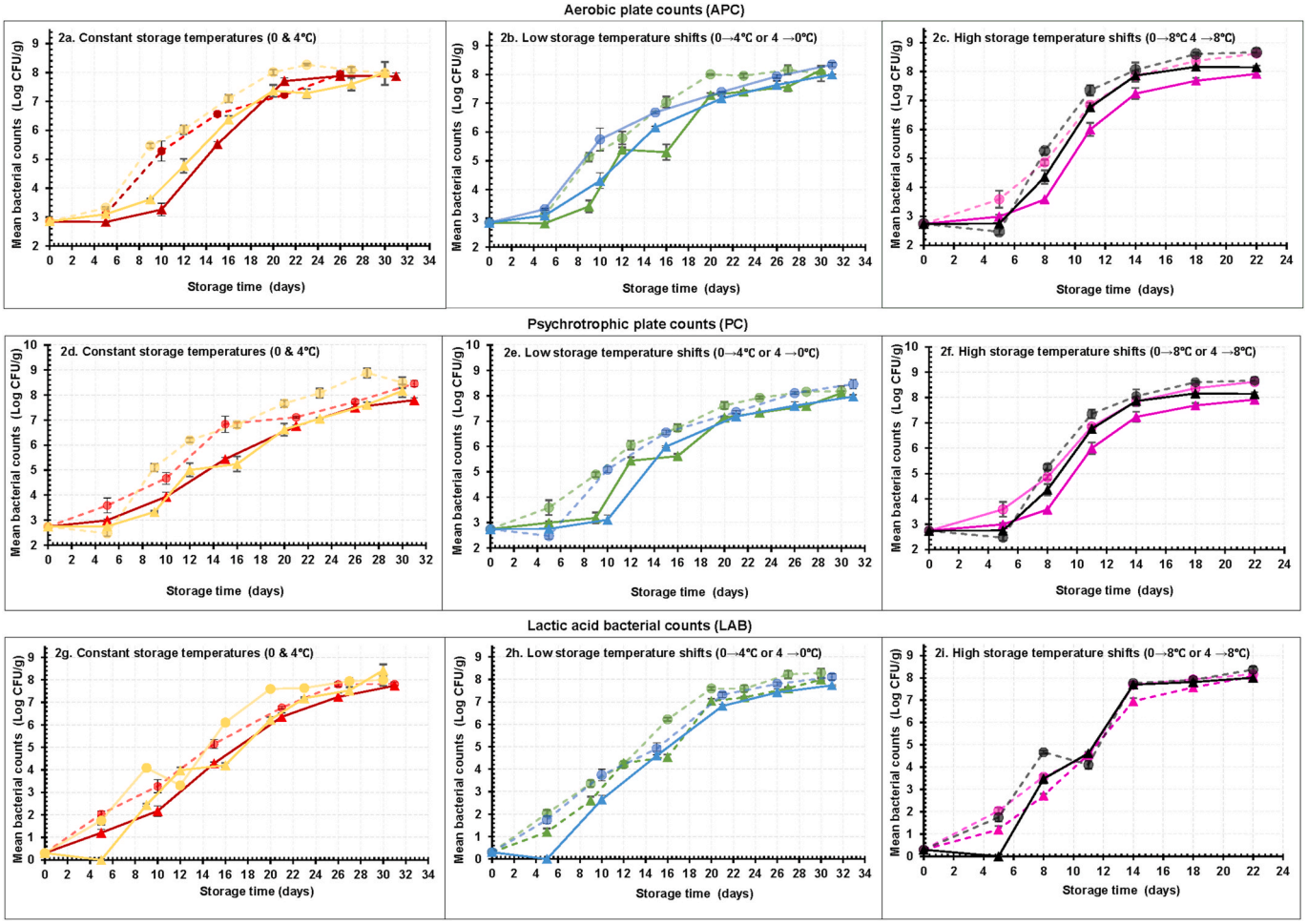

Fig. 2. Evolution of aerobic plate counts (APC), psychrotrophic plate counts (PC), and lactic acid bacterial counts (LAB) in superchilled salmon portions, pretreated with SGS (S) or air (C), vacuum-packed and stored at different temperature scenarios. *Temperature I* (0 or 4 °C) mimics two possible transportation temperatures for the first five days, and *Temperature II* (0, 4 and 8 °C) simulates different storage conditions in the retail segment. Left column (2a, 2 d, 2c); constant storage temperature (*Temperature 1* = *Temperature II*), Mid column (2 b, 2e, 2 h); low-temperature shifts (*Temperature I* = 0 or 4 °C; *Temperature II* = 0 or 4 °C); Right column (2c, 2f, 2i); high-temperature shifts (*Temperature I* = 0 or 4 °C; *Temperature II* = 8 °C). Each point represents the mean values (n = 3), and vertical bars indicate ± SD. Legends: C -Control samples, S-SGS pretreated samples. **C0°C-0°C, S0°C-0°C, C4°C-4°C, S4°C-4°C, C0°C-4°C, S0°C-4°C C4°C-0°C, S4°C-0°C, C0°C-8°C, S0°C-8°C, C4°C-8°C, S0°C-8°C.**

Table 1
Impact of the design variables *Prepackaging treatment* (SGS (S)/Air (C)), *Temperature I* (0 and 4 °C), and *Temperature II* (0, 4 and 8 °C) on microbial growth kinetic parameters (maximum growth rate μ_{max}, lag phase, and final cell concentration Y_{max}) of superchilled vacuum-packed salmon portions. The data are estimated using the primary model of Baranyi and Roberts (1994) using log-transformed bacterial data (n = 3).

Design variables				Aerobic bacteria (APC)					Psychrotrophic bacteria (PC)					Lactic acid bacteria (LAB)				
Pre-packaging	*Temp. I* (°C)	*Temp. II* (°C)	Group ID	μ_{max} (Day^{-1})	Lag phase (Days)	Y_{max} (log CFU/g)	R^2	SE	μ_{max} (Day^{-1})	Lag phase (Days)	Y_{max} (log CFU/g)	R^2	SE	μ_{max} (Day^{-1})	Lag phase (Days)	Y_{max} (log CFU/g)	R^2	SE
S	0	0	S0°C-0°C	0.51 ± 0.01	9.8 ± 0.2	7.86 ± 0.02	1	0.04	0.27 ± 0.02	5.4 ± 0.8	7.75 ± 0.11	1	0.12	0.36 ± 0.03	4.4 ± 1.4	7.69 ± 0.26	0.99	0.27
S		4	S0°C-4°C	0.31 ± 0.07	5.9 ± 2.8	7.86 ± 0.38	0.94	0.51	0.32 ± 0.07	5.8 ± 2.7	7.80 ± 0.35	0.95	0.50	0.37 ± 0.05	2.6 ± 2.1	7.87 ± 0.37	0.98	0.45
S		8	S0°C-8°C	0.88 ± 0.08	6.9 ± 0.3	8.08 ± 0.09	1	0.15	0.74 ± 0.11	6.9 ± 0.7	7.73 ± 0.17	0.99	0.26	0.69 ± 0.06	4.6 ± 0.8	7.89 ± 0.22	0.99	0.29
C		0	C0°C-0°C	0.29 ± 0.07	2.4 ± 3.4	8.12 ± 0.39	0.95	0.51	0.25 ± 0.06	1.1 ± 4.0	8.15 ± 0.47	0.94	0.54	0.32 ± 0.02	0.1 ± 1.1	7.93 ± 0.18	0.99	0.18
C		4	C0°C-4°C	0.37 ± 0.04	3.7 ± 1.3	8.06 ± 0.14	0.99	0.26	0.29 ± 0.02	1.5 ± 1.1	8.12 ± 0.12	0.92	0.19	0.39 ± 0.03	1.2 ± 1.2	8.21 ± 0.19	0.99	0.26
C		8	C0°C-8°C	0.69 ± 0.07	4.8 ± 0.7	8.79 ± 0.17	0.99	0.25	0.55 ± 0.05	3.9 ± 0.7	8.48 ± 0.14	0.99	0.20	0.62 ± 0.12	3.0 ± 1.9	8.16 ± 0.51	0.96	0.64
S	4	0	S4°C-0°C	0.31 ± 0.04	4.8 ± 1.7	7.79 ± 0.19	0.99	0.26	0.64 ± 0.15	9.5 ± 1.3	7.59 ± 0.29	0.98	0.38	0.47 ± 0.07	5.3 ± 1.8	7.56 ± 0.31	0.98	0.42
S		4	S4°C-4°C	0.40 ± 0.05	7.5 ± 1.0	7.62 ± 0.13	0.99	0.23	0.28 ± 0.05	5.4 ± 2.8	8.03 ± 0.46	0.96	0.46	0.38 ± 0.06	3.7 ± 2.8	8.22 ± 0.64	0.96	0.63
S		8	S4°C-8°C	1.00 ± 0.1	6.3 ± 0.4	8.46 ± 0.12	0.99	0.21	0.90 ± 0.14	6.1 ± 0.7	8.07 ± 0.17	0.99	0.29	0.88 ± 0.21	5.0 ± 1.6	7.97 ± 0.52	0.95	0.73
C		0	C4°C-0°C	0.33 ± 0.09	2.5 ± 3.1	7.94 ± 0.32	0.95	0.52	0.42 ± 0.13	4.9 ± 3.1	8.02 ± 0.37	0.94	0.60	0.35 ± 0.03	0.8 ± 1.4	8.10 ± 0.24	0.99	0.27
C		4	C4°C-4°C	0.35 ± 0.05	2.7 ± 1.6	8.10 ± 0.17	0.98	0.31	0.35 ± 0.08	3.1 ± 3.1	8.48 ± 0.39	0.94	0.61	0.39 ± 0.08	1.5 ± 2.9	8.01 ± 0.45	0.95	0.65
C		**8**	**C4°C-8°C**	**0.72 ± 0.07**	**4.4 ± 0.6**	**8.86 ± 0.14**	**0.99**	**0.22**	**0.90 ± 0.18**	**5.3 ± 1.1**	**8.45 ± 0.25**	**0.98**	**0.42**	**0.59 ± 0.19**	**2.3 ± 3.3**	**8.29 ± 0.87**	**0.89**	**1.06**

R^2 coefficient of determination; SE (fit), standard error of fit.

The SGS *Prepackaging treatment* also effectively prolonged the PC lag phase during the constant temperature regime (Fig. 2d) and for the temperature switch (Fig. 2e). Even though psychrotrophic bacteria can adapt to low temperatures (Walker & Betts, 2008), the SGS *Prepackaging treatment* and the temperature shifts made them vulnerable, resulting in a prolonged lag phase. The lag phase of LAB was generally lower than for APC and PC. However, it was longer for SGS-treated samples than for the controls (Fig. 2, Table 1).

Interestingly, the S0°C-8°C and S4°C-8°C samples also showed a significant lag phase extension for APC, PC, and LAB compared to the control samples (Fig. 2c, f, and 2i), reflecting a combined hurdle effect of the initial low temperatures and SGS *Prepackaging treatment* even at *Temperature II* of 8 °C. This result demonstrates the potential of SGS to act as an additional hurdle to prevent microbial growth in retail vacuum-packed salmon exposed to temperature abuse of 8 °C.

No clear pattern for μ_{max} of APC, PC and LAB was demonstrated when growth was established (Table 1). Jakobsen et al. (2022) reported that SGS treatment did not affect μ_{max} of APC. Contradictory SGS *Prepackaging treatment* combined with other hurdles could effectively lower μ_{max} of microorganisms in Atlantic salmon (Abel, Rotabakk, & Lerfall, 2019; Abel, Rotabakk et al., 2019; Hansen, Mørkøre, Rudi, Langsrud, & Eie, 2009; Hansen, Mørkøre, Rudi, Rødbotten et al., 2009; Jakobsen et al., 2022; Lerfall, Jakobsen et al., 2018; Rotabakk et al., 2006; Rotabakk et al., 2008). A linear relationship between dissolved CO_2 in the food matrix and the growth rate of selected spoilage bacteria is established (Devlieghere & Debevere, 2000); however, the three microbial indicators (APC, PC, LAB) used in the present study reflect various bacteria, so it cannot be compared directly.

Recent research reveals that dissolving CO_2 in the food matrix can reshape the microbiota of the product (Abel, Rotabakk, & Lerfall, 2019; Abel, Rotabakk et al., 2019; Hansen, Mørkøre, Rudi, Langsrud, & Eie, 2009; Hansen, Mørkøre, Rudi, Rødbotten et al., 2009; Jakobsen et al., 2022; Lerfall, Jakobsen et al., 2018; Rotabakk et al., 2006; Rotabakk et al., 2008). A high level of dissolved CO_2 in the food matrix causes changes in cell membrane fluidity and permeability, which vary between microorganisms (Kolbeck, Kienberger, Kleigrewe, Hilgarth, & Vogel, 2021); thus, even the growth of highly CO_2-resistant species (like *Photobacterium phosphoreum)* can be effectively prevented (Dalgaard, Mejlholm, & Huss, 1997; Devlieghere & Debevere, 2000). Also, the bacteriostatic effect of CO_2 depends on various factors, including storage temperatures, food processing methods, and packaging conditions (Rotabakk & Sivertsvik, 2012).

The results also give profound knowledge of the effect of temperature regimes during the distribution and storage of superchilled vacuum-packaged salmon portions. Minor temperature shifts cause a massive impact on microbial growth. Recent research also found that constant storage temperatures accounted for a lower bacterial count than the samples exposed to temperature fluctuations (shifts between 0 and 4 °C every 24–48 h) (Yu et al., 2020). Besides, in the present study, the liability is higher for the SGS packaging since the dissolution of CO_2 is higher at lower temperatures; hence, temperature shifts might release the dissolved CO_2 from the fish muscle (Sivertsvik, Jeksrud, Vågane, & Rosnes, 2004). Although some PCs, especially the common spoilers *Photobacterium* spp., and LAB resist CO_2 environments and low temperatures (Tsoukalas et al., 2024; Wong, Ramli, & Son, 2023), saturating the fish muscle with CO_2 and following strict temperature control measures may effectively control their growth by delaying the lag phase. Tsoukalas et al. (2024) demonstrated an inverse proportionality of incubation temperature and growth-reducing effect of CO_2 among *Photobacterium* species. Although the SGS treatment effectively prolonged the lag phase of APC, PC and LAB, all samples reached approximately the same cell density (Y_{max}) at the end of storage (Table 1, Fig. 2).

The present study found a significant correlation between the microbial parameters (r = 0.70 to 0.99). Besides, a significant negative correlation was observed between microbial parameters and pH values (r-values in the range of −0.45 to −0.82) for all samples except for samples stored at 0 °C.

3.3. Chemical quality

Salmon freshness can be evaluated by monitoring ATP degradation products and calculating the K-value (Hong et al., 2017). ATP degradation is catalysed by endogenous and bacterial enzymes in the fish muscle through the intermediate products ADP, AMP, IMP, HxR, and Hx. IMP is recognised for its pleasant taste of salmon, while Hx causes an unpleasant taste and flavour (Hong et al., 2017). In the present study, the raw material had a K-value of 19.45 ± 7.70% (Fig. 3), indicating high-quality raw material (Erikson, Beyer, & Sigholt, 1997).

The K-value was significantly affected by all experimental factors (*Prepackaging treatment*, *Temperature I* and *Temperature II*), and the main discriminant was found to be the *Prepackaging treatment* ($P < 0.001$, $F = 28.7$). On day 5, the SGS samples stored at 0 °C and 4 °C had a K-value of 50.4 ± 7.5% and 49.5 ± 18.3% (Fig. 3), while the control samples had a K-value of 54.6 ± 10.6% (C0°C) and 76.44 ± 1.00% (C4°C).

On day 11, the lowest K-value of 70.4 ± 4.2% was achieved for the S0°C-0°C, although not significantly different from S4°C-4°C, S4°C-0°C, and C0°C-0°C, but significantly lower than all other groups. Overall, the result indicates that SGS prepackaging can maintain the salmon freshness for an extended period compared to the control samples. Furthermore, the SGS pretreated samples are less affected by temperature fluctuations than the control samples, as all SGS samples in the lower temperature regime (S0°C-0°C, S4°C-4°C, S4°C-0°C) were comparable to the freshness of the C0°C-0°C group. In contrast, the control groups exposed to temperature fluctuations demonstrated significantly higher K-values.

The H-value can be used as a spoilage indicator as it reflects the accumulation of Hx (Daskalova, 2019). In the present study, the H-value was significantly affected by all experimental factors: *Prepackaging treatment* ($P < 0.001$, $F = 13.9$), *Temperature I* ($P < 0.001$, $F = 13.5$) and *Temperature II* ($P < 0.001$, $F = 7.3$) (Fig. 3). On day 5, the control samples stored at 4 °C reached an H-value of 16.9 ± 0.6%, significantly higher than for the control group stored at 0 °C and the SGS groups (One-way-ANOVA, $P = 0.040$). Significantly differences among the experimental groups were found for the H-values (One-way-ANOVA $P < 0.001$ at day 11, of which the groups S0°C-0°C, S4°C-4°C, C0°C-0°C had an H-value in the range of 15–18% (Fig. 3a), only significantly lower than S4°C-8°C which reached an of approximately 22% (Fig. 3c), numerically higher than the samples stored at constant temperature.

In the present study, the overall development of the ATP degradation products increased with increased storage temperatures in control samples. On the other hand, the implementation of SGS *Prepackaging treatment* demonstrates a protective effect against IMP loss during minor temperature shifts (0 and 4 °C). However, prolonged exposure of samples to uncontrolled temperatures or an elevation in storage temperature (8 °C) is associated with a detrimental impact on IMP concentration, accompanied by a concurrent increase in HxR and Hx formation.

The Hx accumulation, which increases the H-value, was dependent on microbial activity, of which bacterial enzymes that catalysis Hx development (Huss, 1995; Mohan, Ravishankar, Gopal, & Kumar, 2009). In the present study, positive correlations were found between Hx concentration and all microbial parameters in the range of 0.43–0.99, except in C0°C-8°C where a negative correlation (r = −0.37) was found between Hx formation and LAB.

Biogenic amines (BA) can be formed due to decarboxylase enzymes of spoilage microorganisms and represent as fish spoilage indicators (Biji, Ravishankar, Venkateswarlu, Mohan, & Gopal, 2016; Yamanaka, Shiomi, & Kikuchi, 1989). Thus, in the present study, the concentration of cadaverine, spermidine, spermine, tryptamine, and tyramine was measured on day 11.

The levels of spermidine, tryptamine, and tyramine were, in general, low in all samples (<2 μg/g), with no significant differences among the experimental groups (One-way-ANOVA, P>0.05). The spermine level

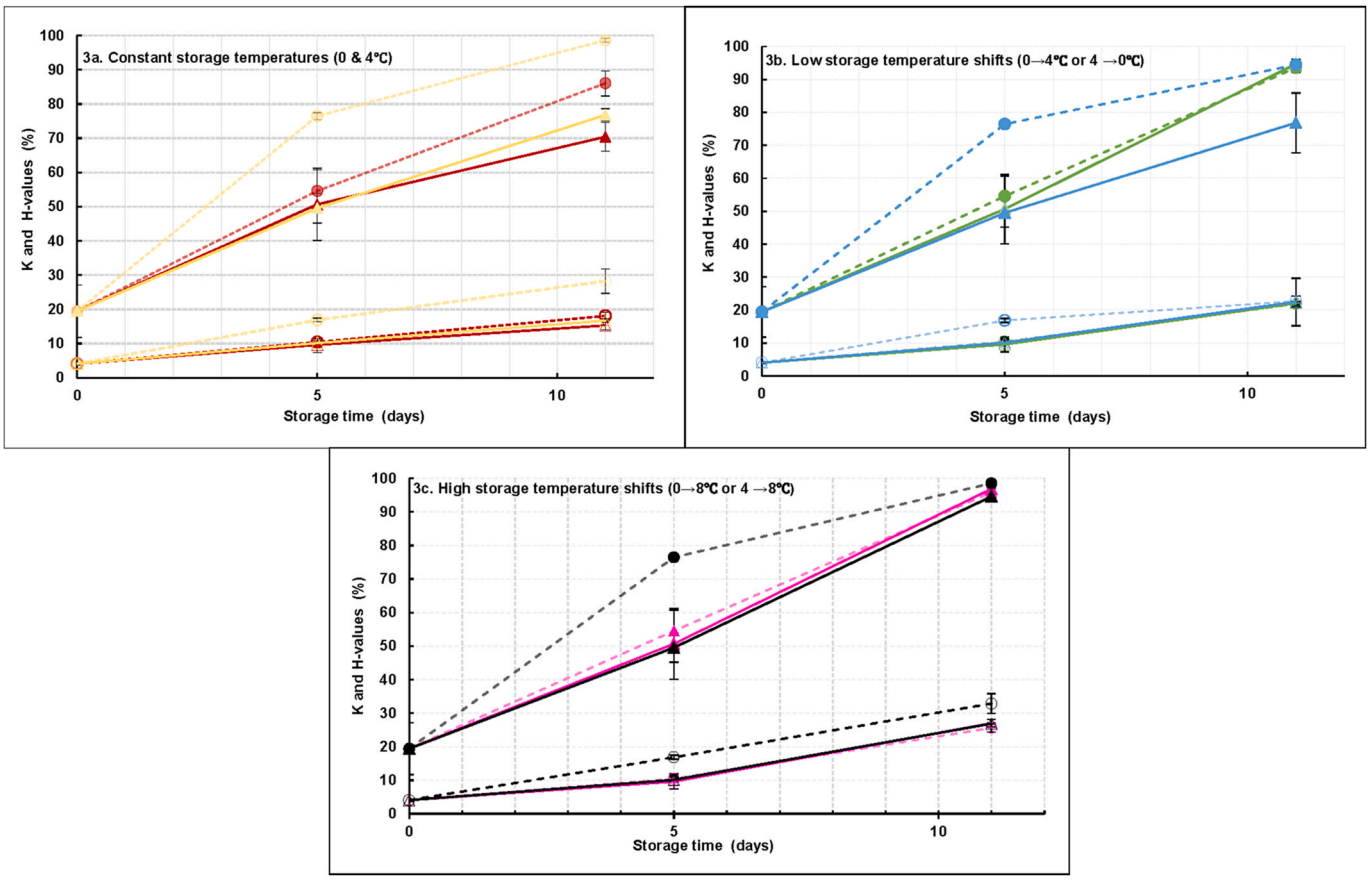

Fig. 3. Freshness (K values, closed symbols) and Spoilage (H values, open symbols) of superchilled vacuum-packed pre-rigor salmon portions pretreated with SGS (S) or air (C), and stored at different temperature scenarios. *Temperature I* (0 or 4 °C) mimics two possible transportation temperatures for the first five days, and *Temperature II* (0, 4 and 8 °C) simulates different storage conditions in the retail segment. The values at each sampling point represent mean values (n = 3) with standard error. Legends: C -Control samples, S-SGS pretreated samples.

was also generally low, ranging from 3.7 to 7.6 μg/g. The cadaverine concentration was significantly affected by the experimental design, with *Temperature II* ($P < 0.001$, $F = 100.7$) as the main discriminant, followed by *Prepackaging treatment* ($P < 0.001$, $F = 78.4$) and *Temperature I* ($P < 0.001$, $F = 54.7$).

Cadaverine concentration was significantly highest in the C4°C-8°C samples (One-way-ANOVA, $P < 0.00$), at a concentration of 85.1 ± 9 μg/g. Also, C0°C-8°C samples were numerically higher than the other samples, reaching 14.5 ± 4.7 μg/g. In the respective SGS-treated samples, the cadaverine concentrations were 7.9 ± 3.0 μg/g for the S4°C-8°C samples and 1.5 ± 0.3 μg/g for the S0°C-8°C samples. Thus, the cadaverine concentration was approximately ten times higher in the control samples than in the SGS-pretreated samples.

Yamanaka et al. (1989) also observed that cadaverine concentration increased with increased temperature in rainbow trout and contradicting observations in salmon. The present experimental results also contradicted the previous research on saithe muscle, which stated that a high CO_2 environment increased cadaverine production (Lerfall, Thomassen, & Jakobsen, 2018). Since BA formation is a spoilage indicator, their development must be increased with the storage time and proportional to the specific microorganism growths with decarboxylase activities (Yamanaka et al., 1989; Zarei, Najafzadeh, Enayati, Pashmforoush, & Chamran, 2011). Nevertheless, on day 11, the bacterial count of all other samples would be around 5 log CFU/g or even lower, except for the samples stored at 8 °C. Hence, BA should be quantified on further sampling points to get a clear overview of the applied hurdles on amine production at the storage of 0 and 4 °C.

3.4. Fillet colour

Fillet colour is among Atlantic salmon's most essential quality traits (Kiessling, Espe, Ruohonen, & Mørkøre, 2004; Robb, Kestin, & Warriss, 2000). In the present study, the fillet lightness (L*) was unaffected by the design variable *Prepackaging treatment* ($P > 0.055$, Table 2). CO_2 has previously been reported to affect salmon fillet lightness. However, the literature is inconsistent, showing both fillet bleaching (Barnett et al., 1982) and darkening (Chan et al., 2021; Choubert & Baccaunaud, 2006; Hansen, Mørkøre, Rudi, Rødbotten et al., 2009) and no significant effects (Jakobsen et al., 2022), supporting the present L*-values. Independent of *Prepackaging treatment*, both *Temperature I* and *Temperature II*

Table 2
The impact of the design variables *Prepackaging treatment* (SGS/Air), storage *Temperature I* (0 and 4 °C), and *Temperature II* (0, 4 and 8 °C) on colourimetric properties (CIE Lab) of superchilled and vacuum-packaged Atlantic salmon loins.

Parameter	*Pre-packaging*	Average value	*Temperature I*	Day 5_{value}	*Temperature II*	Day 11_{value}
L*	Control (Air)	62.5 ± 1.6	0 °C	63.3 ± 1.2	0 °C	64.1 ± 1.7
					4 °C	62.2 ± 1.0
					8 °C	64.4 ± 1.0
						[1] P > 0.141
			4 °C	61.8 ± 1.6	0 °C	62.7 ± 1.1
					4 °C	61.6 ± 0.4
					8 °C	63.6 ± 1.0
				[a] P = 0.006		[1] P > 0.063
	SGS (CO_2)	62.0 ± 1.2				
			0 °C	62.4 ± 0.7	0 °C	63.4 ± 1.2
					4 °C	62.5 ± 0.4
					8 °C	61.6 ± 0.5
						[1] P > 0.90
			4 °C	61.7 ± 1.4	0 °C	63.6 ± 1.2
					4 °C	61.7 ± 1.6
					8 °C	62.8 ± 0.5
				[a] P > 0.097		[1] P > 0.300
	[b]GLM pre-pack	***P > 0.055***	***[b]GLM temperature I***	***P < 0.001***	***[b]GLM temperature II***	***P = 0.032***
a*	Control (Air)	17.6 ± 1.1	0 °C	17.3 ± 1.0	0 °C	18.0 ± 0.8[a]
					4 °C	17.3 ± 0.5[b]
					8 °C	18.0 ± 0.5[a]
						[1] P = 0.026
			4 °C	18.0 ± 1.1	0 °C	19.5 ± 0.4
					4 °C	18.3 ± 0.8
					8 °C	18.7 ± 0.6
				[a] P > 0.065		[1] P > 0.187
	SGS (CO_2)	16.8 ± 1.3	0 °C	16.9 ± 1.1	0 °C	18.4 ± 1.1
					4 °C	17.0 ± 2.0
					8 °C	15.3 ± 1.5
						[1] P > 0.057
			4 °C	16.6 ± 1.4	0 °C	19.5 ± 0.4[a]
					4 °C	14.8 ± 2.0[b]
					8 °C	15.8 ± 1.0[ab]
				[a] P > 0.527		[1] P = 0.032
	[b]GLM pre-pack	***P = 0.001***	***[2]GLM temperature I***	***P > 0.446***	***[b]GLM temperature II***	***P = 0.004***
b*	Control (Air)	17.4 ± 2.7	0 °C	17.5 ± 2.6	0 °C	20.1 ± 0.5
					4 °C	19.1 ± 0.9
					8 °C	20.3 ± 0.4
						[1] P > 0.119
			4 °C	17.4 ± 3.0	0 °C	20.4 ± 1.0[ab]
					4 °C	20.0 ± 0.2[b]
					8 °C	20.4 ± 0.1[a]
				[1] P > 0.873		[1] P = 0.038
	SGS (CO_2)	17.0 ± 2.7	0 °C	17.4 ± 2.6	0 °C	20.4 ± 0.1
					4 °C	19.9 ± 0.6
					8 °C	18.7 ± 1.6
						[1] P > 0.170
			4 °C	16.6 ± 2.8	0 °C	20.4 ± 0.1
					4 °C	17.7 ± 2.3
					8 °C	20.3 ± 0.4
				[a] P > 0.403		[a] P > 119
	[b]GLM pre-packaging	***P = 0.045***	***[b]GLM temperature I***	***P > 0.147***	***[b]GLM temperature II***	***P > 0.110***

[a] Significant difference between groups by one-way ANOVA. Significant different superscript letters (a,b) indicate significant variations between groups at storage *Temperature II* by one-way ANOVA combined with Tukey HSD at $P < 0.05$.
[b] Main effect analyses by General Linear Modeling (GLM) using the design variables *Pre-packaging*, storage *Temperature I*, and *Temperature II* as factors, $P < 0.05$.

significantly impacted the fillet lightness ($P < 0.001$, $F = 15.5$ and $P = 0.032$, $F = 3.6$, respectively). Generally, increasing the storage temperature gave, on average, slightly darker fillets than those stored at 0 °C. Although the observed differences were low (<1 colour unit), indicating a negligible impact on consumer preferences, complementary studies to investigate potential adverse sensory effects and oxidation will be beneficial.

Both the fillet redness (a*) and yellowness (b*) were influenced by the design variable *Prepackaging treatment* ($P = 0.001$, $F = 11.5$ and $P = 0.045$, $F = 4.2$), but differently affected by the storage temperature (Table 2). Whereas the fillet yellowness was unaffected by both *Temperature I* and *Temperature II* ($P > 0.147$ and $P > 0.110$, respectively), the fillet redness was highly affected by *Temperature II* ($P = 0.004$, $F = 6.0$) but not by *Temperature I* ($P > 0.446$). The effect of *Temperature II* on the fillet redness (significantly lower redness of samples stored at 8 °C as compared to those stored at 0 and 4 °C) might be attributed to changes in the fillet surface as affected by microbial growth parameters and spoilage in general (Cheng & Sun, 2015).

4. Conclusion

SGS *Prepackaging treatment* and *Temperature II* were the main discriminants delaying the proliferation of microorganisms in superchilled vacuum-packed salmon portions. Moreover, this study demonstrated that SGS *Prepackaging treatment* could obtain increased microbial stability at 4 °C as obtained for the control samples at 0 °C and delay microbial growth during temperature fluctuations in the cold chain. The impact of the design factors on the fillets freshness and appearance was minor. However, increased temperature generally gave darker and less reddish fillets than those following an optimal cold chain. Overall, the results demonstrate that SGS technology can be combined with super chilling and vacuum packaging to preserve high-quality pre-rigor filleted salmon portions.

CRediT authorship contribution statement

Revathy Gurusamy: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Jørgen Lerfall:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Bjørn Tore Rotabakk:** Writing – review & editing, Conceptualization. **Anita Nordeng Jakobsen:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The Research Council of Norway financially supports this work through the research project “Concept development of full-scale soluble gas stabilisation (SGS) technology for seafood” (project number 294641). The authors would like to thank the technical staff at the food science division at NTNU for their practical help during the experiments.

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