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Effect of salt on CO_2 solubility in salmon (*Salmo salar L*) stored in modified atmosphere

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ABSTRACT

Salt and CO_2 in the form of modified atmosphere (MA) packaging are often used forms of preservation of seafood, as they both are known for their ability to reduce spoilage and health risks. However, little is known regarding interaction between the two when applied to seafood. Hence this experiment investigated CO_2 solubility in salmon injected with various brines and packaged using MA packaging or soluble gas stabilization (SGS) followed by MA packaging. Regardless of packaging method, increasing NaCl concentration decreased the absorbed CO_2 concentration, as seen from analysis of head space composition, calculations of Henry's constant, and absorbed amount of CO_2 within the product. However, the effect was only significant after long storage, as no effect of NaCl was observed in the samples before MA packaging in retail trays. Furthermore, use of SGS significantly increased amount of absorbed CO_2 as compared to regular MA packaging. The effect of SGS outnumbered the negative effect of NaCl, thus making the use of both NaCl and CO_2 possible without losing effect of either.

1. Introduction

Multiple parameters are being used by food manufacturer in order to preserve foods and increase shelf life. Amongst these are temperature-controlled storage, alterations of pH, addition of salts, and use of modified atmosphere (MA) amongst others (Albarracin et al., 2011).

Traditionally salting is one of the most widely used forms of preservation (Kim et al., 2017). This is due to the preservative effect of salt which is ascribed to its ability to reduce water activity of foods (Mariutti and Bragagnolo, 2017). Several studies have proven that the addition of salt to seafood products reduces bacterial growth (Gram and Huss, 1996), decreases water activity, and influences enzyme activity (Mariutti and Bragagnolo, 2017) prompting a potential for increased shelf life. Additionally, salt is often used as a flavor enhancer. On the other hand, salt has been reported as a potent pro-oxidant, causing lipid oxidation in meat and seafood products (Aubourg and Ugliano, 2002; Shimizu et al., 2009), resulting in production of off flavors and odors (Mariutti and Bragagnolo, 2017). Furthermore, although salting is considered one of the most effective preservation methods, it does not ensure microbial safety on its own (Kim et al., 2017).

Multiple papers including a recent review by Bouletis et al. (2017) have shown that CO_2-rich MA packaging can reduce microbial growth and increase the shelf life of many foods as compared to air- or vacuum packaging. This effect have also been observed with various fish species (Abel et al., 2019; Speranza et al., 2009; Torrieri et al., 2006). It has been proven that the inhibitory effect achieved by MA packaging is directly proportional to the concentration of dissolved CO_2 in the food product (Devlieghere, Debevere and van Impe, 1998a, 1998b).

CO_2 is generally highly soluble in both muscle and fatty tissues and even more so in pure water (Gill, 1988). Several factors have the ability to influence the uptake of CO_2, including pH, lipid content, and lipid type (Abel et al., 2018; Gill, 1988; Jakobsen and Bertelsen, 2004), water content (Sivertsvik et al., 2004a,b), and importantly salt content. The latter have been shown to decrease the solubility of CO_2 in various aqueous solutions (Jakobsen et al., 2009; Rotabakk, 2013; Rumpf et al., 1994). These findings are often being used to estimate a similar effect in seafood products. However, the relationship is not straight forward. Although electrolytes like salt decreases solubility of CO_2 in aqueous solutions, it is known that salt influences water uptake in muscle foods. It has previously been shown in Gallart-Jornet et al. (2007) that low brine salt concentrations (less than 20%) led to an uptake of water in salmon fillets, an effect known as salting-in. In contrast high brine salt

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Table 1
Experimental design and response variables. MA = modified atmosphere, SGS = soluble gas stabilization.

Design variables	Levels
Brine	0% and 10% w/v NaCl-brine, 0% and 10% w/v NaCl-brine carbonated
Packaging method	MA packaging (all brine types) or SGS followed by MA packaging (non-carbonated samples)
Sampling times	2, 6, 11, 20, 24, 31, 44, 57, 68, 76, 92, 116, and 140 h after MA packaging
Response Variables	**Analyses**
Quality	Composition, headspace gas composition, headspace gas volume, pH.
Tested samples	n = 5 for each group, at each sampling point

concentrations (above 20% or in case of dry-salting) led to significant loss of water, known as salting-out. These effects are due to the degree of protein denaturation, caused by the salt, influencing the water holding capacity (Barat et al., 2002). With CO_2 being highly soluble in water, the increased water content could potentially counter the decrease in solubility caused by salting. The salinity effect on CO_2 solubility in solid food matrices is less referenced (Chaix et al., 2014) and thus less understood. To the best of our knowledge, only a single study have investigated the effect of varying salt concentrations on the CO_2 transfer properties (Acerbi et al., 2016). The aim of this study is thus to expand the knowledge of solubility of CO_2 in solid foods and to study the influence of salt concentration on CO_2 uptake in seafood in various MA systems.

2. Materials and methods

A two-factor storage experiment was conducted, the factors being brine type (NaCl-brine 0% or 10% w/v, or carbonated NaCl-brine 0% or 10% w/v) and packing method (MA packaging or soluble gas stabilization (SGS) followed by MA packaging) as summarized in Table 1.

2.1. Raw material

Farmed Atlantic salmon fillets (*Salmo salar L.*) were procured from Salmar ASA (Frøya, Norway). The samples had been filleted pre-rigor by Salmar ASA. Samples were obtained 3 days after slaughter and stored on ice in fridge (3.8 ± 1,6 °C) until processing. Tail, belly- and backflaps were removed before use.

A surplus of brine was prepared from tap water (approximately 8 °C) and NaCl in a 10% NaCl ratio (w/v). One portion was transferred to an airtight stainless-steel keg fitted with a CO_2 cannister and pressurized to 2 bar. The keg was vigorously shaken for approximately 20 min to ensure CO_2 saturation (4.2 ± 2.4 °C). Water-brines were prepared similarly. Brines were prepared the day before processing and stored in fridge overnight.

2.2. Salting

Trimmed fillets were individually fed into a brine injector (PSM-57-2.5 ZD, Dorit DFT Fleischereimaschinen GmbH, Ellwangen, Germany) fitted with recirculating brine. The injection pressure was set to 3.2 bar. The carbonated brine was continuously purged with CO_2 throughout the injection process. The fillets were subsequently portioned into equal sizes of 82.5 ± 1.7 g (height approximately 3 cm).

2.3. Packaging

All samples were packaged immediately after injection. SGS samples (half of the total amount of samples) were placed in batches (n = 13) on trays (C2325-1C, Færch Plast, Holstebro, Denmark) in high-barrier pouches (425 × 650 mm PA/PE sous vide pouch, Maske AS, Trondheim, Norway, filling degree approx. 17%). The pouches were filled with pure CO_2 in excess using a chamber machine (Webomatic SuperMax s3000, Webomatic, Bochum, Germany). Meanwhile, the MA samples were stored under vacuum in high-barrier pouches (135 × 180 mm PA/PE sous vide pouch, Maske AS, Trondheim, Norway). All the samples were stored for 16 h at 3.7 ± 1.0 °C. The next day all samples were repacked into 300 mL semi-rigid crystalline polyethylene terephthalate (CPET) trays (C2125-1B, Færch Plast, Holstebro, Denmark) using a semi-automatic tray sealing packaging machine (TL250, Webomatic, Bochum, Germany). All trays were equipped with an absorbent before sealing. During packaging, the air was evacuated (final vacuum pressure of 25 mbar) and flushed with the pre-set MA gas mixture 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). Food grade CO_2 and N_2 were mixed to 60% CO_2 balanced with N_2 using a gas mixer (MAP Mix 9000, Dansensor, Ringsted, Denmark). Oxygen transmission rate (OTR) was 66–78 $cm^3 \times 25\ \mu m\ x\ m^{-2}\ x\ 24\ h^1\ x\ bar^1$ at 23 °C for the tray, 2.5 $cm^3 \times 40\ \mu m\ x\ m^{-2}\ x\ 24\ h^1\ x\ atm^1$ at 23 °C for the cover film, and 50 $cm^3\ x\ m^{-2} \times 24\ h^1 \times bar^1$ at 23 °C for the high-barrier pouches. Packaging resulted in a sample filling degree of approximately 1:3.

After packaging, the trays were stored at 2.2 ± 1.4 °C for up to 7 days.

A total of six sample parameter combinations were used. These were (in shorthand) $control_W$ (non-carbonated water + MA packaging), $control_S$ (non-carbonated 10% NaCl-brine + MA packaging), $carb_W$ (carbonated water + MA packaging), $carb_S$ (carbonated 10% NaCl-brine + MA packaging), SGS_W (non-carbonated water + SGS), and SGS_S (non-carbonated 10% NaCl-brine + SGS)

2.4. Chemical analysis

2.4.1. Water, lipid, and NaCl content

Water content was determined gravimetrically by drying the samples for 24 h at 105 °C (ISO.6496, 1983). Lipids were extracted and the total amount calculated as described by Bligh and Dyer (1959). NaCl content was measured from warm water extracts using a multimeter (Hach HQ40d, Hach, CO, USA) equipped with a chloride ion selective electrode (Hach Intellical™ ISECL181, Hach, CO, USA). Measurement of chloride ions is a good indicator of NaCl content in brines and salt-added and thus used despite the inaccuracy in measuring muscle samples.

2.4.2. pH

pH was measured in the center of the salmon muscle at each sampling point using a multimeter (Hach HQ40d, Hach, CO, USA) equipped with a puncture pH electrode (Hach Intellical™ PHC108, Hach, CO, USA).

2.4.3. Headspace gas composition

The headspace gas composition (% O_2 and CO_2) was measured using an oxygen and carbon dioxide analyzer (Checkmate 9900 analyzer, PBI-Dansensor, Ringsted, Denmark) as described by Abel et al. (2018). The gas composition was measured at every sampling point.

2.4.4. Headspace gas volume

The headspace gas volume (mL) was assessed 2, 6, 11, 20, 24, 31, 44, 57, 68, 76, 92, 116, and 140 h after packaging. Measurements were done by submerging the trays under water and measuring the buoyancy force using a texture analyzer (Stable Micro System Ltd, TA-XT plus, Godalming, UK) as described by Rotabakk et al. (2007). The trays were submerged at a rate of 2 mm/s for 30s and held submerged for 30s to stabilize. Buoyancy force was measured every 2s a total of 10 times. Averaged measurements were used for the data analyses. All measurements were corrected for atmospheric pressure at time of packaging and at time of measurement. The product density was measured to be 1080

kg/m^3.

The concentration of absorbed CO_2 is related to changes in package volume as described by Rotabakk et al. (2007):

$$C_{CO_2}^{t=\infty} = \frac{1,000 \cdot P\left(V_g^{t=0} - V_g^{t=\infty}\right) \cdot MwCO_2}{R \cdot T \cdot W_f} \quad (1)$$

where $C_{CO_2}^{t=\infty}$ is the total CO_2 (ppm) absorbed by the product, P is absolute pressure (Pa), V_g is gas volume (m^3) at start and at equilibrium, $MwCO_2$ is the molecular weight of CO_2, R is the gas constant, T is the absolute temperature (K), and W_f is the weight of the product (kg). The change in volume is solely ascribed to the dissolvement of CO_2, as any gas consumption or production due to microorganisms as well as the changes in partial pressure of N_2 and O_2 is neglectable (Sivertsvik et al., 2002).

According to Henry's law, once a sample has reached equilibrium with the surrounding gas, the amount of CO_2 in the headspace is proportional to the amount of CO_2 absorbed in the sample (Schumpe et al., 1982):

$$P_{CO_2}^{t=\infty} = H_{CO_2,p} \cdot C_{CO_2}^{t=\infty} \quad (2)$$

where $P_{CO_2}^{t=\infty}$ is the equilibrium partial 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).

All calculations rely on the assumption that the ideal gas law is valid since the packages are believed to be a closed system and that the amount of CO_2 is kept constant.

Henry's constant is dependent on temperature and the composition of the product used, as different components have different absorption potential. In the given design variations in product parameters included NaCl and amount of added CO_2. The amount of CO_2 added in the form of SGS or carbonation of the brine has the potential to cause pH changes due to dissociation (Sivertsvik et al., 2002). However, in the present study, CO_2 did not significantly alter pH and is therefore not expected to influence Henry's constant of the samples. NaCl content, on the other hand, is highly influential on CO_2 absorption and Henry's constant, thus two different approximations to Henry's constant were calculated.

2.5. Statistics

Statistical analyses, including outlier test and analysis of variance (ANOVA) were performed using minitab 18 (Minitab, Coventry, UK). Outlier testing was performed using Grubbs outlier test at level $p < 0.05$. GLM was performed using Tukey's HSD test at level $p < 0.05$. Data are given as mean ± standard deviation (SD) unless otherwise stated.

3. Results and discussion

NaCl and CO_2 are often used forms of preservatives for seafood, as they both are well known for their abilities to hamper microbial growth and chemical deterioration. At the same time, both use of NaCl and CO_2 has the potential to affect properties of a product.

3.1. Product characterization

The NaCl content of the raw salmon fillets was 0.7 ± 0.1% with no significant difference between the two batches used ($p = 0.283$). In the present study salmon fillets where injected with either water or NaCl-brine resulting in significantly altered NaCl content (to an average of 0.5 ± 0.1% and 1.2 ± 0.1% for 0% and 10% brine injected samples, respectively, $p < 0.001$) caused either by introduction of NaCl or by dilution by added water. Different application of CO_2 (control, carbonation or SGS) showed no significant influence on NaCl content ($p = 0.785$–0.951). The NaCl-brine injected samples showed significantly higher water content, compared to water injected samples (72 ± 1%, 66 ± 1%, respectively $p < 0.001$), regardless of no initial differences in water content between the raw materials used ($p = 0.183$). This effect is due to NaCls ability to bind water by causing swelling of the proteins in muscle foods (Böcker et al., 2008; Offer and Trinick, 1983). Similar changes have previously been reported due to pH variations (Martínez-Alvarez and Gómez-Guillén, 2006). CO_2, as used in the MA packaging in the present study, has the ability to change pH of the product due to dissociation into carbonic acid when reacting with water, thus acidifying the product. However, no such changes were observed in the present study ($p = 0.320$) (data not shown). Similar results have been reported by Silbande et al. (2018) amongst others.

Table 2
Changes in CO_2 concentrations [%] from packaging to equilibrium. Superscript letters indicate statistically significant differences at level $\alpha = 0.05$. $control_W$ (non-carbonated water + MA packaging), $control_S$ (non-carbonated NaCl-brine + MA packaging), $carb_W$ (carbonated water + MA packaging), $carb_S$ (carbonated NaCl-brine + MA packaging), SGS_W (non-carbonated water + SGS), and SGS_S (non-carbonated NaCl-brine + SGS).

Sample	ΔCO_2-equilibrium concentration [%]
$control_W$	18.0 ± 0.3^a
$control_S$	17.6 ± 0.4^a
$carb_W$	15.2 ± 0.3^b
$carb_S$	14.6 ± 0.2^b
SGS_W	3.5 ± 0.4^c
SGS_S	2.3 ± 0.2^d

Table 3
CO_2 concentration in the samples, adjusted for water and liquid lipid content. Concentrations based on volume changes indicate changes in CO_2 concentration during storage. Concentrations based on Henry's constant considers the entire process from raw fillet to equilibrium after storage. The difference between the two prior relates to CO_2 uptake during processing, preceding retail packaging. Superscript letters indicate significant difference at level $\alpha = 0.05$ within each row. CO_2 concentration in the control samples were used for the calculation of Henry's constant.

Sample	CO_2 conc. based on volume changes - Storage [Pa ppm^{-1}]	CO_2 conc. based on Henry's constant - Raw to equilibrium [Pa ppm^{-1}]	CO_2 uptake preceding packaging [Pa ppm^{-1}]
$control_W$	1519 ± 71^a	1519 ± 71^d	–
$control_S$	$1401 \pm 74^{c,b}$	1401 ± 74^e	–
$carb_W$	$1500 \pm 15^{b,a}$	1659 ± 13^c	159 ± 27^b
$carb_S$	1337 ± 57^c	$1467 \pm 7^{d,e}$	130 ± 59^b
SGS_W	307 ± 39^d	2087 ± 15^a	1798 ± 12^a
SGS_S	130 ± 48^e	1863 ± 6^b	1733 ± 40^a
p-values	<0.001	<0.001	<0.001

3.2. Solubility of CO_2

CO_2 uptake can be assessed in multiple ways. In the present experiment headspace CO_2 concentration was measured throughout the experiment and differences between initial packaging mixture concentration and equilibrium concentration indicates the degree of CO_2 uptake in the sample (Table 2).

The headspace CO_2 method only relates to the uptake of CO_2 in the final retail packaging, not the steps preceding. Thus, smaller changes in CO_2 uptake during storage indicates higher uptake have been achieved before packaging. The present experiment showed that both use of carbonation and SGS significantly decreases delta concentration in the samples CO_2 ($p < 0.001$), indicating significantly higher uptake prior to packaging. No differences were observed between treatments with respect to water or NaCl-brine injected with the exception of SGS-samples.

Alternatively, CO_2 concentration in the sample itself at equilibrium can be estimated based on volume changes as described in equation (1) under the assumption that CO_2 is only absorbed during packaging

(imitated by the control-samples in the present experiment). Equilibrium CO_2 concentrations in the control samples were significantly different ($p = 0.040$) at 1401 ± 74 CO_2 ppm and 1520 ± 71 CO_2 ppm, for NaCl-brine injected and water injected, respectively (Table 3).

Generally, the use of Henry's constant is considered the most universal way of presenting solubility data as it allows for comparisons between samples. As mentioned above, unlike the amount of absorbed CO_2, Henry's constant is independent of the gas composition of the packaging gas and is estimated on both the concentration of CO_2 in the packaging headspace as well as in the samples themselves. Henry's constant is highly product specific, as various components have different absorption potential (Gill, 1988). Thus, in order to standardize the results, adjustments must be made based on composition. It has previously been shown that CO_2 is dissolved in the water and *liquid* lipid content of a sample (Abel et al., 2018), making these components the bases for adjustment. Combined water and lipid content were 77 ± 2% and 72 ± 2% for NaCl-brine and water injected, respectively. Consequently, adjusted approximations to Henry's constant were 42.9 ± 2.6 for water injected samples and 52.4 ± 3.0 Pa ppm^{-1} for NaCl-brine injected samples. Sivertsvik et al. (2004b) reported Henry's constant for a variety of raw fish fillets at 0 °C to be in the range of 41.8 ± 4.7 Pa ppm^{-1} to 49.1 ± 5.2 Pa ppm^{-1}, and for salmon at 4 °C to be 57.9 ± 4.5 Pa ppm^{-1}, thus similar to the findings in the present study.

The established Henry's constants enable calculations of CO_2 uptake for all the samples as stated in equation (2). This measure describes the uptake throughout the entire process from raw fillet to equilibrium in the retail packages. The results show a significant difference between all samples, both with respect to source of CO_2 and brine injection (Table 3).

As mentioned, the CO_2 concentration calculated based on volume change only signifies the changes during storage in retail packaging, whereas the CO_2 concentrations calculated based on Henrys's constant considers the entire process. The differences between the two measures indicate CO_2 uptake before the final packaging in retail packages (Table 3).

Regardless of method for determining solubility of CO_2, all analyses found NaCl to be negatively influencing absorbed amount of CO_2 ($p < 0.001$–0.040). This is seen both from the gas composition analysis as well as calculated CO_2 concentration, based either on volume changes and Henry's constant. This is ascribed to changes in the water fraction caused by the increased electrolyte concentration (Chaix et al., 2014). As seen from the water content analysis (section 3.1) increased NaCl concentration increases water content of the samples, thus making ground for increased solubility of CO_2. However, as the increased amount of water is bound and not free, as shown by Schumpe et al. (1982), it is no longer available for CO_2 uptake. This is an effect known as salting out (Battino and Clever, 1966; Schumpe et al., 1982). Similar observations have been made in multiple studies regarding aqueous solutions, including studies by Duan and Sun (2003), Raji et al. (2019), and Rumpf et al. (1994). Still, little research have been published regarding this impact of salts on the solubility of CO_2 in real food products, and especially in seafood (Chaix et al., 2014).

Furthermore, regardless of brine type, all samples showed final CO_2 concentration to decrease in the order SGS > carbonation > control. This is in agreement with previous studies which have found SGS to significantly increase CO_2 concentration in seafood when compared to MA packaging (Abel et al., 2019; Rotabakk et al., 2008; Sivertsvik, 2000). To the extent of our knowledge, no studies have previously been using carbonated brine. Schirmer et al. (2009) packed fresh salmon in salt and acid brines with a small 100% CO_2 headspace thereby obtaining a carbonized brine. The experiment did not report solubility data, however reports were made that "*[…]all CO_2 dissolved in the product within hours after packing, leaving only small bubbles (d <3 mm) in the package corners*" (Schirmer et al., 2009) indicating higher solubility when using the brining method compared to regular MA packaging. This agrees with the findings of the present experiment.

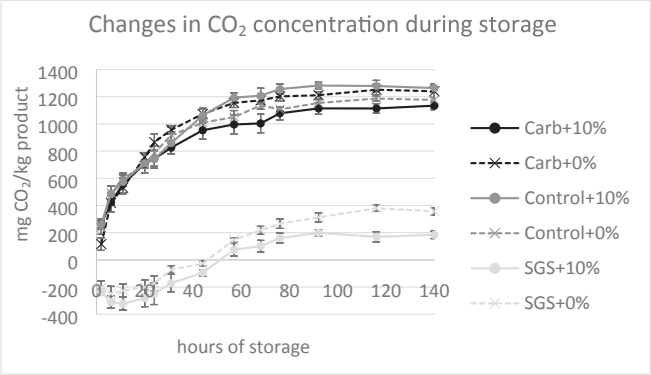

Fig. 1. Changes in CO_2 concentration in the samples [mg CO_2/kg of product] estimated based on volume change between 2 and 140 h of storage in MA packaging. Black = samples injected with carbonated brines, dark grey = control samples, light grey = SGS-treated samples. Dotted lines = 0% w/v NaCl-brine injected, solid line = 10% w/v NaCl-brine injected samples.

The calculated CO_2 uptake preceding final packaging show no significant influence of the NaCl-brine ($p = 0.138$–0.392) (Table 3) unlike that seen for the final concentration. This shows that the effect of NaCl concentration on CO_2 absorption is only perceptible over longer periods of storage. This might be explained by the gradient nature of CO_2 transfer; large initial CO_2 concentration differences between the sample and its surrounding drives the absorption of CO_2 which gradually decreases with decreasing difference. The findings of the present study show that NaCl only significantly influences CO_2 uptake towards the stage of equilibrium. The short period applied for the carbonation or SGS-treatment, is therefore not sufficient for NaCl to be influential. This is supported by the fact that all samples needed between 57 ($control_S$, $carb_W$) and 68 ($carb_S$, SGS_S, $control_W$, SGS_W) hours to reach equilibrium (Fig. 1). To the best of our knowledge, no previous experiments have investigated this effect.

The nature of CO_2 absorption and desorption is further highlighted by the uptake pattern observed for the SGS treated samples. Regardless of brine type, SGS-samples showed a decrease in CO_2 concentration during the first 12 h of storage followed by an increase until equilibrium was reached (Fig. 1). During SGS-treatment the outermost parts of the samples become saturated with CO_2 whereas the concentration decreases towards the center. Therefore, when the sample is transferred to a MA gas mix containing 60% CO_2 then the concentration in the sample surface would be higher than the gas mix. This causes a two-way diffusion from the sample outerparts; one towards the low concentration in the center and a desorption of CO_2 from the sample to the headspace, as explained by the decrease observed (Fig. 1). As far as we are aware no study has previously reported this development. Rotabakk, Lekang, and Sivertsvik (2010) estimated the desorption rate of CO_2 from SGS-treated chicken breast fillets when stored in atmospheric air, after SGS treatment, before packaging in MA, however, as only end-point measurements were performed comparison is not feasible.

To summarize, from an industrial point of view, although NaCl negatively influences CO_2 uptake, the effect is not more prominent that the effect of SGS vs. MA packaging. This means that higher CO_2 concentrations can be achieved using both NaCl and SGS, rather than regular MA packaging, which is considered the industrial practice. This could give rise to a potentially better microbial inhibition and thereby longer shelf life, due to the combined effect of NaCl and CO_2.

Declaration of competing interest

None.

CRediT authorship contribution statement

Nanna Abel: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Bjørn Tore Rotabakk:** Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision. **Jørgen Lerfall:** Conceptualization, Investigation, Writing - review & editing, Supervision.

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