Effect of salt on CO₂ solubility in salmon (*Salmo salar L*) stored in modified atmosphere

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**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 spoilage and health risks. However, little is known regarding interaction between the two when applied to seafood. Hence this experiment investigated CO₂ 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 CO₂ concentration, as seen from analysis of head space composition, calculations of Henry’s constant, and absorbed amount of CO₂ 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 CO₂ as compared to regular MA packaging. The effect of SGS outnumbered the negative effect of NaCl, thus making the use of both NaCl and CO₂ possible without losing effect of either.

**Keywords:**

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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 CO₂ being highly soluble in water, the increased water content could potentially counter the decrease in solubility caused by salting. The salinity effect on CO₂ 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 CO₂ transfer properties (Acerbi et al., 2016). The aim of this study is thus to expand the knowledge of solubility of CO₂ in solid foods and to study the influence of salt concentration on CO₂ 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 (Froya, 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 CO₂ canister and pressurized to 2 bar. The keg was vigorously shaken for approximately 20 min to ensure CO₂ 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-57.2Z, 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 CO₂ 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 CO₂ 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 (CPEF) 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 CO₂ and N₂ were mixed to 60% CO₂ balanced with N₂ using a gas mixer (MAP Mix 9000, Dansensor, Ringsted, Denmark). Oxygen transmission rate (OTR) was 66–78 cm² × 25 μm × m⁻² × 24 h⁻¹ × bar⁻¹ at 23 °C for the tray, 2.5 cm³ × 40 μm × m⁻² × 24 h⁻¹ × atm⁻¹ at 23 °C for the cover film, and 50 cm³ × m⁻² × 24 h⁻¹ × 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) controlW (non-carbonated water + MA packaging), controlS (non-carbonated 10% NaCl-brine + MA packaging), carbW (carbonated water + MA packaging), carbS (carbonated 10% NaCl-brine + MA packaging), SGSW (non-carbonated water + SGS), and SGSS (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 Intellitem™ 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 Intellitem™ PHC108, Hach, CO, USA).

#### 2.4.3. Headspace gas composition

The headspace gas composition (% O₂ and CO₂) 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 Rotabak 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/m³.

The concentration of absorbed CO₂ is related to changes in package volume as described by Rotabakk et al. (2007):

$$C_{CO₂}^{v_{\infty}} = \frac{1.000 \cdot P \cdot (V_{x}^{\infty} - V_{x}^{v_{\infty}}) \cdot MW_{CO₂}}{R \cdot T \cdot W_t}$$

(1)

where $C_{CO₂}^{v_{\infty}}$ is the total CO₂ (ppm) absorbed by the product, $P$ is absolute pressure (Pa), $V_x$ is gas volume (m³) at start and at equilibrium, $MW_{CO₂}$ is the molecular weight of CO₂, $R$ is the gas constant, $T$ is the absolute temperature (K), and $W_t$ is the weight of the product (kg). The change in volume is solely ascribed to the dissolution of CO₂ as any gas consumption or production due to microorganisms as well as the changes in partial pressure of N₂ and O₂ is neglectable (Sivertsvik et al., 2002).

According to Henry’s law, once a sample has reached equilibrium with the surrounding gas, the amount of CO₂ in the headspace is proportional to the amount of CO₂ absorbed in the sample (Schumpe et al., 1982):

$$P_{CO₂}^{v_{\infty}} = H_{CO₂} \cdot P \cdot C_{CO₂}^{v_{\infty}}$$

(2)

where $P_{CO₂}^{v_{\infty}}$ is the equilibrium partial pressure of CO₂ in the headspace gas (Pa), $H_{CO₂}$ is the temperature dependent Henry’s constant for CO₂ 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 CO₂ 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 CO₂. The amount of CO₂ 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, CO₂ 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 CO₂ 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). 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 CO₂ 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 CO₂ has the potential to affect properties of a product.

3.1. Product characterisation

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 CO₂ (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). CO₂, 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.

3.2. Solubility of CO₂

CO₂ uptake can be assessed in multiple ways. In the present experiment headspace CO₂ concentration was measured throughout the experiment and differences between initial packaging mixture concentration and equilibrium concentration indicates the degree of CO₂ uptake in the sample (Table 2).

| Sample     | ΔCO₂-equilibrium concentration [%] |
|------------|-----------------------------------|
| non-carb   | control                           |
| CO₂ conc.  | Storage [Pa ppm⁻¹] | CO₂ conc. based on Henry’s constant - Raw to equilibrium [Pa ppm⁻¹] | CO₂ uptake preceding packaging [Pa ppm⁻¹] |
| non-carb   | control                           |
| carbon     | 1519 ± 71² | 1519 ± 71² | – |
| carb       | 1401 ± 74³ | 1401 ± 74³ | – |
| carb       | 1500 ± 15⁴ | 1500 ± 15⁴ | – |
| carb       | 1337 ± 75⁴ | 1337 ± 75⁴ | – |
| SGS        | 307 ± 39⁴ | 2087 ± 15⁴ | 1798 ± 12⁴ |
| SGS        | 130 ± 48⁴ | 1863 ± 6⁴ | 1733 ± 40⁴ |
| p-values   | <0.001  | <0.001  | <0.001 

Table 3

CO₂ concentration in the samples, adjusted for water and liquid lipid content. Concentrations based on volume changes indicate changes in CO₂ 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 CO₂ uptake during processing, preceding retail packaging. Superscript letters indicate significant difference at level $a = 0.05$ within each row. CO₂ concentration in the control samples were used for the calculation of Henry’s constant.

The headspace CO₂ method only relates to the uptake of CO₂ in the final retail packaging, not the steps preceding. Thus, smaller changes in CO₂ 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 CO₂ ($p < 0.001$), 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, CO₂ concentration in the sample itself at equilibrium can be estimated based on volume changes as described in equation (1) under the assumption that CO₂ is only absorbed during packaging

\[ C_{CO₂}^{v_{\infty}} = \frac{1.000 \cdot P \cdot (V_{x}^{\infty} - V_{x}^{v_{\infty}}) \cdot MW_{CO₂}}{R \cdot T \cdot W_t} \]
(imitated by the control-samples in the present experiment). Equilibrium CO₂ concentrations in the control samples were significantly different (p = 0.040) at 1401 ± 74 CO₂ ppm and 1520 ± 71 CO₂ 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 CO₂, Henry’s constant is independent of the gas composition of the packaging gas and is estimated on both the concentration of CO₂ 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 CO₂ 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 for NaCl-brine and water 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 CO₂ 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 CO₂ and brine injection (Table 3).

As mentioned, the CO₂ concentration calculated based on volume change only signifies the changes during storage in retail packaging, whereas the CO₂ concentrations calculated based on Henry’s constant considers the entire process. The differences between the two measures indicate CO₂ uptake before the final packaging in retail packages (Table 3).

Regardless of method for determining solubility of CO₂, all analyses found NaCl to be negatively influencing absorbed amount of CO₂ (p < 0.001–0.040). This is seen both from the gas composition analysis as well as calculated CO₂ 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 sample, thus making ground for increased solubility of CO₂. 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 CO₂ 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 CO₂ in real food products, and especially in seafood (Chaix et al., 2014).

Furthermore, regardless of brine type, all samples showed final CO₂ concentration to decrease in the order SGS > carbonation > control. This is in agreement with previous studies which have found SGS to significantly increase CO₂ 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% CO₂ headspace thereby obtaining a carbonized brine. The experiment did not report solubility data, however reports were made that “[...all CO₂ 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.

The calculated CO₂ 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 CO₂ absorption is only perceptible over longer periods of storage. This might be explained by the gradient nature of CO₂ transfer; large initial CO₂ concentration differences between the sample and its surrounding drives the absorption of CO₂ which gradually decreases with decreasing difference. The findings of the present study show that NaCl only significantly influences CO₂ 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 (controls, carb₀) and 68 (carb₁₀, SGS₀, control₁₀, SGS₁₀) hours to reach equilibrium (Fig. 1). To the best of our knowledge, no previous experiments have investigated this effect.

The nature of CO₂ 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 CO₂ 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 CO₂ whereas the concentration decreases towards the center. Therefore, when the sample is transferred to a MA gas mix containing 60% CO₂ 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 CO₂ 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 CO₂ 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 CO₂ uptake, the effect is not more prominent that the effect of SGS vs. MA packaging. This means that higher CO₂ 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 CO₂.

**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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