The influence of lipid composition, storage temperature, and modified atmospheric gas combinations on the solubility of CO_2 in a seafood model product

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Abstract

The demand for tasty, convenient, fresh seafood products is continually increasing. This stresses the need for processing methods that can prolong the otherwise short shelf life of seafood. A well-studied method is the use of modified atmosphere packing. However, research into the use of modified atmosphere packaging for seafood with varying lipid composition is limited. Thus, in this experiment the effect of lipid profile, storage temperature, and the gas composition of the modified atmosphere on the solubility of CO_2 in a seafood model product was investigated. The temperature dependent Henry's constants for the various compositions showed that the physical state of the lipids clearly influenced the solubility of CO_2 in the model products, with liquid fat leading to a similar solubility of CO_2 as water, while CO_2 only being minimally dissolved in solid fats.

Keywords

Modified atmosphere packaging; seafood model product; lipid composition; storage temperature; gas composition

1 Introduction

Recent socio-economic development has led to an increase in time pressure brought about by work and pastime activities as well as increasing amounts of single person and/or small households (Speranza et al., 2009). Extensive campaigning has increased consumers awareness of the benefits of fish and seafood, however many feel a lack of abilities and experience with preparing seafood. This has tremendously increased the demand for convenient, tasty meal products based on fresh fish (Hansen et al., 2015; Mendes and Gonçalves, 2008). Fresh seafood has a limited shelf life as a result of multiple factors often specific to these particular foods, including high post mortem pH, presence of large amounts of unsaturated fatty acids (affected by fish species), and presence of autolytic enzymes (Gram and Huss, 1996; Sivertsvik et al., 2002). The nature of seafood stresses the need for improved preservation methods that allow extension of shelf life. Multiple technologies are being used for this purpose, and modified atmosphere packaging (MAP) in combination with refrigeration has become one well-established method (Lambert et al., 1991). Several studies have found MAP to extend shelf life for several days compared to air storage depending on species and temperature (Powell and Tamplin, 2012; Sivertsvik et al., 2003; Speranza et al., 2009; Torrieri et al., 2006; Tsironi and Taukis, 2010; Özogul et al., 2004). The spoilage of fish begins as soon as the fish dies and is ascribed to a series of reactions where degradation is caused by bacteria (Speranza *et al.*, 2009).

MAP often uses a mixture of oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2), and the prolongation of shelf life is often ascribed to the bacteriostatic effect of CO_2 (Genigeorgis, 1985). A certain amount of CO_2 has to be dissolved into the food in order to inhibit bacterial growth (Gill and Penney, 1988), and it has been found that the inhibition obtained is proportional to the concentration of dissolved CO_2 (Devlieghere et al., 1998a, b). CO_2 is generally highly soluble in both muscle and fatty tissues and even more so in pure water (Gill, 1988). Several factors will however influence the uptake; including pH, lipid content, lipid type (Gill, 1988; Jakobsen and Bertelsen, 2004), salt content (Rumpf et al., 1994), amount of initial CO_2 in the gas mixture (Devlieghere *et al.*, 1998a), and water content (Sivertsvik et al., 2004). Several studies have found that solubility of CO_2 in muscle food could be estimated based on the water content alone, for instance in raw fish (Sivertsvik *et al.*, 2004), chicken (Rotabakk et al., 2010), and meat (Gill, 1988). However disagreements exists as Fava and Piergiovanni (1992) concluded the solubility of CO_2 in fresh meat and meat products estimated based on water content alone was misleading. Probably the most important factor influencing the solubility of CO_2, is temperature. This effect has been extensively studied, amongst other by Gill (1988), Mendes et al. (2011), and Rotabakk (2013). Previous results generally agree that increasing temperatures will decrease the solubility of CO_2 in muscle tissues, just as it is known from water (Caroll et al., 1991). The relationship between temperature and solubility of CO_2 in fatty samples is not as simple. Rotabakk (2013) found the solubility of CO_2 in liquid salmon oil to be similar to that in water. However, when Gill

(1988) examined the solubility of CO_2 in fat from lamb, beef, and pork, he found the solubility to increase with increasing temperatures, to a certain point, unlike that seen in water or muscle tissue. The point at which increasing temperatures led to a decrease in solubility of CO_2 was different for the different fat sources. These results shows that the effect of temperature on the solubility of CO_2 in fatty tissue is more complex than other samples and the mechanism is not well understood (Gill, 1988; Jakobsen and Bertelsen, 2006). Besides shelf life, solubility of CO_2 also influences the risk of packages collapsing (Rotabakk and Sivertsvik, 2012). Thus, understanding and manipulating the solubility of CO_2 in various products is important from both a packaging and shelf life point of view. This underlines the fact that different foods, with different compositions have to be treated differently. Thus, in order to optimize the industrial use of MAP it is essential to obtain knowledge regarding the specific product and processing of interest in relation to the solubility of CO_2.

Even though solubility of CO_2 in various products is well studied, comparison between studies are often difficult. Furthermore, investigation of the solubility of CO_2 in seafood products with varying lipid composition is limited. The aim of this study is thus to expand the knowledge of the solubility of CO_2 in seafood by studying the effect of lipid phase composition of a fish mince model product, temperature, and initial gas mix.

2 Materials and methods

A three-factor storage experiment was conducted, the factors being lipid composition (mix of stearic acid, oleic acid and an eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) mix), storage temperature (0°, 8°, and 20°C), and packaging gas CO_2 concentration (35, 50, and 70%, balanced with N_2).

2.1 Production of fish model product

Frozen mince of silver smelt (*Argentina silus)* were purchased from Norwegian Seafood Company. The fish mince had a water content of 69.8±0.5% and lipid content of 1.2±0.1%. The lipid phase was primarily made up of saturated- and monounsaturated fatty acids (6% C14:0, 37% C16:0, 6% C18:0, 20% C18:1, 12% C20:1, 15% C22:1). The fish mince was thawed at 4°C for 24 hours prior to being mixed in a bowl chopper (Blixer 6, Robot Coupe, France) at 20 000rpm. Salt (0.5%) was added prior to the addition of 6.5% of potato starch, 20% of skimmed milk (0.1% lipid), and 18% of the lipid mixtures. The composition of the model product was calculated in order to keep the total amount of added liquid and lipids constant. The lipids were stearic acid as free fatty acid (Stearic Acid ≥95%, Sigma-Aldrich, St. Louis, Missouri, USA), oleic acid as free fatty acid (Oleic Acid 90%, Sigma-Aldrich, St. Louis, Missouri, USA) and an EPA/DHA oil mix as triglycerides (EPAX 4535 TG/N, Epax Norway, Ålesund, Norway). Stearic- and oleic acid had high purity (97% C18:0 and 93% C18:1 combined with 6% C18:2,

respectively) whereas the marine oil had a more diverse fatty acid profile with two major constituents and multiple minors (51% C20:5, 37% C22:6, 1-5% C18:4, C20:4, C22:1, C22:5). The lipids were mixed according to Figure 1, and will hereafter be denoted as "recipe 1", "recipe 2", etc. The lipids were chosen based on melting points, in order to investigate the influence of the different phases of lipids on the solubility of CO_2.

A total mixing time of 150s was applied. A control sample, recipe 8, was produced with extra milk, but without the addition of lipids. Each recipe of mince was produced in two batches and mixed by hand for 30s. The mince was stuffed in plastic casing (Ø=60mm, L=30-40cm), closed with metal clips and heat-treated at 100˚C for 1 hour. After the heat treatment, the mince product was cooled in the fridge at +4±1˚C for 2-4 hours, prior to being frozen at -23±1°C until packaging.

2.2 Packaging

The mince product was thawed for 48 hours at 4°C prior to being sliced in portions. The samples were packaged (101.3g ± 4.1g) in 300ml semi rigid crystalline polyethylene terephthalate (CPET) trays (C2125-1B, Færch Plast, Holstebro, Denmark) using an automatic packaging machine (TL250, Webomatic, Bochum, Germany). This gave a degree of filling of approximately ⅓. The atmosphere was evacuated (final vacuum pressure of 18mbar) and subsequently flushed with the gas mixture prior to adhering the top film of a 40µm combination of polyethylene (PE), ethylene vinyl alcohol (EVOH), polyamide (PA), and polyethylene terephthalate(Topaz B-440 AF, Plastopil, Almere, The Netherlands). Food grade CO_2 and N_2 was mixed using a gas mixer (MAP Mix 9000, Dansensor, Ringsted, Denmark) to obtain pre-set packaging gas mixtures of 35% CO_2, 50% CO_2, or 70% CO_2 all balanced with N_2. Hereafter referred to as 35/65, 50/50, and 70/30. Oxygen transmission rate (OTR) was 66-78cm^3 x 25µm/m^2 x 24h^1 x bar^1 at 23˚C for the tray and 2.5cm^3 x 40µm/m^2 x 24h^1 x atm^1 at 23˚C for the cover film.

After packaging, the trays were stored at 0˚C (0.6±0.7˚C), 8˚C (7.8±0.5˚C), or 20˚C (20.0±0.3˚C) respectively, for 7 days.

2.3 Water content, lipid content, and fatty acid composition

The water content of all the groups was determined gravimetrically by drying the samples for 24 hours at 105˚C (ISO.6496, 1983). Lipids were extracted and total amount calculated gravimetrically from the mince product as described by Bligh and Dyer (1959). Eight samples were taken from each recipe and each sample was divided into two; one for analysis of water content and one for analysis of lipid content. The fatty acid composition was analyzed as fatty acid methyl esters in the lipid extracts from the fish mince samples and in the pure lipids. Fatty acid methyl esters were analyzed using gas chromatography (GC) (Agilent 6850 GC-system, Waldbronn, Germany) equipped with a flame

ionization detector (FID) set to 310°C, on a polyethylene glycol column (HP-INNOWax, Agilent, Waldbronn, Germany 30m × 250µm × 0.25µm). Helium was used a carrier gas and the oven temperature was set to 210°C. Preparation of methyl esters of the samples was conducted as described by Metcalfe et al. (1966).

2.4 Differential scanning calorimetry analysis

The pure fats and samples recipe 1 through 8 were used for differential scanning calorimetry (DSC) analysis. DSC was performed at a cooling rate of - 5 °C min^{-1} over a temperature range from 20 °C to -70 °C and then from -70 °C to + 70 °C at a heating rate of + 5 °C min^{-1} on a DSC1 (Mettler Toledo, Schwerzenbach, Switzerland). Aluminum crucibles (40 µl) were filled with sample (29.57 ± 1.96 mg for stearic acid, 30.54 ± 1.36 mg for oleic acid and 31.17 ± 1.60 mg for the model products) and sealed. An empty crucible was used as reference.

The enthalpy changes during cooling and heating were recorded. A rescan of previously scanned samples was performed to identify irreversible reactions. The results were obtained by StarE software version 14.0 (Mettler Toledo, Schwerzenbach, Switzerland).

2.5 Headspace gas analysis

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). 20ml of the headspace gas was collected with a syringe after intrusion of the top film. Before measurement of the composition, a rubber septum (Nordic Supply, Skodje, Norway) was placed onto the top foil in order to avoid rupture and introduction of false atmosphere. The gas compositions was measured in empty trays immediately after packaging and in all trays at the end of the storage period.

2.6 Headspace gas volume

The headspace gas volume (mL) was assessed every day from day 1 through 7 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 2mm/s for 30s and held submerged for 30s to stabilize. Buoyancy force was measured every 2s a total of 10 times. An average of the measurements was used for the further analyses. All measurements were corrected according to the actual atmospheric pressure. The product density was measured to be 1.08kg/m^3.

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

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

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).

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):

$$P_{CO_2}^{t=\infty} = H_{CO_2,p} \cdot C_{CO_2}^{t=\infty}$$

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). The absorption of CO_2, and thus Henry's constant is dependent on the composition of the product used, as different components have different absorption potential. Comparison of Henry's constant for different foods can thus only be carried out using adjustments to standardize the obtained results. In the present study, three different adjustment methods were tested with the use of different assumptions (absorption only in the water content, absorption in water- and total lipid content, or absorption in the water and liquid lipid content). Content of liquid lipids were calculated based on storage temperature and theoretical melting point. Obtained Henry's constants were divided by the amount of absorptive content under each of the three assumptions.

2.7 Statistics

Statistical analyses, including outlier test, analysis of variance (ANOVA) and general linear modelling (GLM) were performed using minitab 17.0 (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

GLM analyses showed all parameters (storage temperature, lipid profile, storage time and packaging gas combination) as well as all of the interactions effects to be of significant influence on the amount of estimated dissolved CO_2 and Henry's constant ($p<0.001$). Only looking at the main effects, the main discriminant factor for amount of estimated dissolved CO_2 was found to be packaging gas combination ($F=116999$) followed by storage temperature ($F=30747$), lipid type ($F=3653$), and finally storage time ($F=1041$). Despite showing interaction effects, all parameters were tested in combinations using ANOVA.

The model used to estimate absorption of CO_2 into the model product is based on the assumption that true flexible packages were used (Rotabakk et al., 2007). In the present study, truly flexible packages were not obtainable and thus semi rigid trays were used. Even though the original study assume true flexibility in the set-up the model was tested using semi rigid trays, and good correlation were seen between estimated absorption and absorption calculated based on Henry's constant. In Rotabakk et al., (2007) some problems with under pressure were seen when using high degrees of filling (48% or higher) combined with high initial CO_2 levels (above 75%). In the present study, the degree of filling was approximately 33% and initial CO_2 levels never rose above 70%, thus both parameters lower than the critical values from the original study by (Rotabakk et al., 2007). Therefore, despite the breach of the underlying assumptions, the results are still valid under the given circumstances.

3.1 Raw material

3.1.1 Lipid content, water content, and fatty acid composition

Silver smelt was chosen as raw material based on its excellent freeze-thaw stability, good water holding capacity, and low natural lipid content (Hellevik et al., 2005). These properties allowed for a stable raw material that would not change significantly during the storage period. One of the focuses of the current study was to investigate the influence of added lipids on the solubility of CO_2. By choosing a raw material with a low initial lipid content, the findings of the experiment is representative of the added lipids and not to the fish itself.

No significant difference in total lipid content was found in recipe 1 through 6. Recipe 7 exhibited a visible lipid loss that resulted in a significant lower lipid content ($p<0.001$) as compared to recipe 1 through 6. Analysis of the sum of lipid and water content showed the eight recipes to be separated into two significantly different groups. Group one contains recipe 1 through 6 and group two contains recipe 7 and 8. The recipes within each group was not significantly different from each other (Table 1).

The experiment was designed to obtain model products with a predetermined fatty acid profile, but analyses showed small deviation between planned and actual composition (Figure 1 and Table 1). The deviation is probably due to inaccuracies during production, as well as impurities in the added lipids as seen from the lipid profiles of the pure oils (Table 1).

3.1.2 Differential scanning calorimetry analysis

DSC was performed in order to establish melting point/ranges of all samples. The results of multiple DSC scans showed stearic acid to have a large change in energy in accordance with a phase transition, confirming the expected melting point to be 75-80˚C (data not shown). The marine oil was a mix of multiple fatty acids, with the main constituents being EPA and DHA. This was evident in the analyses

that showed a wider range of phase transitions temperatures, as was expected with the sample being a mixture. The entire range of the melting temperatures was outside the range of storage temperatures, and thus not influential on the model product during storage. The pure oleic acid was found to contain small amounts of linoleic acid in addition to the oleic acid. These findings was confirmed by the DSC analysis, which showed two separate peaks at -8˚C and +18˚C (Figure 2A), respectively. The +18˚C peak representing the oleic acid, is very close to the storage temperature of 20˚C. Furthermore, the scans shows that the full phase transition has not taken place before reaching approximately 25˚C. This shows that the oleic acid would not be in a complete liquid state during storage, even at a storage temperature of 20˚C.

3.2 Solubility of CO_2 in the fish model product

The relationship between the recipes and amount of dissolved CO_2 is rather complex, but certain observations apply in general. The samples containing a pure marine oil (recipe 7) is always insignificantly different from the control sample (recipe 8) with few exceptions. Results from 70/30 (Figure 3) are good representatives to the trends seen for samples packed with 50/50 and 35/65 (data not shown). The solubility of CO_2 in fish has previously been found to be significantly dependent on the total amount of water and lipid (Sivertsvik *et al.*, 2004). This correlates with recipe 7 and 8 having no significant difference in sum content of lipid and water, while being significantly different from the other recipes (Table 1 and Figure 3). It is believed that higher water and lipid content would lead to higher amounts of dissolved CO_2 (Sivertsvik *et al.*, 2004). In the present study, samples containing least water and lipid (recipe 7 and 8) had the significantly highest amount of dissolved CO_2, regardless of packaging gas combination, storage time, and storage temperature ($p<0.001$). This indicates that the *physical state* of the liquids (water and lipid) is more influential on the solubility than the *amount*. This is confirmed by the finding of Devlieghere *et al.* (1998a) indicating that CO_2 mostly dissolves in liquid lipid. This, in turn, explains why the samples containing 50% marine oil in the mixture (recipe 2 and 3) was not significantly different (Figure 3), as the main influence on solubility is from the liquid marine oil. This is further strengthened by the fact that the samples without liquid lipids (types 5, 6 and 1) reached the lowest levels of dissolved CO_2 (Figure 3). The use of oleic- and stearic acid was included to investigate this effect. Oleic- and stearic acid was chosen based on the differences in the excepted melting points, +13˚C and +69˚C, respectively (McMurry and Simanek, 2007). It was believed that melting points above and below storage temperatures would result in different amounts of dissolved CO_2 depending on the storage temperature. However, the samples containing pure oleic- or stearic acid (recipe 6 and 5) showed no differences in dissolved amounts, regardless of temperature. Looking at the DSC results, a change in energy was observed in in the range from -5 to +15˚C, indicating a phase transition in this range (Figure 2C). This transition is ascribed to be the content of water. The analysis

262 was performed using a temperature increase rate of 5˚C/min. This high rate of increase explains the
263 displacement of the water transition from the expected value of 0˚C. When looking at the results for
264 recipe 6, the water-phase transition peak is shouldered by a smaller peak at around +16˚C, resulting
265 from the content of oleic acid (Figure 2D). This shows that the lipids within the sample would be liquid
266 or at least semi-liquid when stored over longer periods at 20˚C. This should lead to a potential for
267 increased uptake of CO_2 contradictory to the results observed for amount of dissolved CO_2 in the
268 current study. The reason for this inconsistency is not understood.
269 A better way to compare the solubility of CO_2 in different samples is to use Henry's constant. Henry's
270 constant is calculated using the concentration of CO_2 in the headspace gas *and* in the sample. Thus,
271 unlike amount of absorbed CO_2, Henry's constant is independent of the gas composition of the
272 packaging gas (Table 2). It has been customary to standardize Henry's constants of samples based on
273 the water content alone. This is done based on the assumption that CO_2 dissolves mainly in the water
274 phase (Meredith et al., 2014; Rotabakk et al., 2010). However, as mentioned, it has been found that
275 the solubility of CO_2 is dependent on the amount of both water and lipid (Jakobsen et al., 2009;
276 Rotabakk, 2013; Sivertsvik et al., 2004). Furthermore, (Gill, 1988) established that whereas the
277 solubility in water decreased with increasing temperatures, the solubility of CO_2 in lipid increased with
278 increasing temperatures up to a certain point depending on the fat source. This effect was ascribed to
279 the melting of the lipids. This indicates that the CO_2 is more prone to dissolve in liquid lipids rather
280 than solid lipids. It is usual to assume that Henry's constant for a food product can be calculated based
281 on Henry's constant for pure water and the water content of the product (Meredith et al., 2014). When
282 doing so, we find that the theoretical Henry's constant is a good approximation to the one measured
283 (data not shown) for all samples only containing solid lipids. On the other hand, samples containing
284 liquid lipids, that is all samples with some percentage of marine oil (recipe 2, 3, 4, and 7) and samples
285 with oleic acid when stored at 20˚C (recipe 1 and 6), show the theoretical value to be a poor
286 representative of the actually measured value of Henry's constant. This indicates that the proper way
287 to present Henry's constant is to adjust for the content of water and *liquid* lipid. This further highlights
288 the influence of the relationship between lipids, melting points and storage temperatures, unlike what
289 was seen in the results for absorbed amount of CO_2 (Figure 3). All mentioned adjustment methods are
290 presented in Table 2. The choice of adjustment is further supported by the fact that adjustment based
291 on the water content or the sum of water and total lipids together reveals questionable results with
292 Henry's constants lower than those of water (30.3 Pa/ppm at 0˚C, 39.9 Pa/ppm at 8˚C, and 57.6
293 Pa/ppm at 20˚C, respectively (Caroll *et al.*, 1991)).
294 Samples stored at 0˚C revealed Henry's constants ranging from 31.9±2.9 Pa/ppm to 49.0±2.2 Pa/ppm,
295 recipe 7 and 6, respectively (Figure 4). The results clearly shows the temperature dependency of
296 Henry's constant, with increasing Henry's constant with increasing temperature. Recipe 1, 2, 3, 4, 5, 7,

297 and 8 all show high linearity with changes in temperature (R^2=0.89-1.00), however this is not the case
298 for recipe 6 (R^2=0.62). Increasing the temperature from 0 to 8°C, as expected, shows a significant
299 increase in Henry's constant. A further temperature increase does not result in further increase in
300 Henry's constant. This is due to the partial melting of the lipids changing the product composition. This
301 correlates perfectly with total content of water and liquid lipid (Table 1), showing that the solubility of
302 CO_2 in food is highly influenced by the composition of the fatty acids. Previous studies have reported
303 Henry's constants for multiple food products including several fish species. Sivertsvik *et al.* (2004)
304 found 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 to
305 49.1±5.2 Pa/ppm, which is similar to the majority of the results obtained in the present study. Fish
306 samples are not normally stored at temperatures as high as 8˚ or 20˚C, and results are therefore not
307 presented for comparison.

308 The CO_2 level in the headspace immediately after packing was found to be 69.3±0.4, 50.2±0.3, and
309 35.6±0.3% CO_2, for 70/30, 50/50, and 35/65, respectively. The O_2 level was low for all samples
310 (0.1±0.2%), indicating that sufficient evacuation of the headspace was achieved.
311 As expected the packaging gas composition had a highly significant influence on the equilibrium CO_2
312 concentration in the headspace. Similar results was seen for dissolved CO_2 (Table 2). Solubility of CO_2
313 increased for all combinations of parameters with increasing initial CO_2 concentration. These findings
314 is supported by several other studies, including Rotabakk (2013), Rotabakk et al. (2008), and Sivertsvik
315 et al. (2004). A similar relationship as of packaging gas combination can be seen for the influence of
316 storage temperature. An increase from 0˚C to 8˚C or 20˚C significantly lowered the amount of dissolved
317 CO_2 and increased Henry's constants in all samples, regardless of other parameters. These findings
318 agrees with results of previous studies including Rotabakk (2013).
319 Packaging gas composition had a significant influence on how the samples developed throughout the
320 storage period ($p<0.001$). Samples packed in both 50/50 and 70/30 showed a significant increase in
321 amount of dissolved CO_2 between measurements at 24 and 48 hours of storage ($p<0.04$), with few
322 exceptions. For the samples packed with 35/65, an increase in dissolved CO_2 concentration was not
323 observed until between 48 and 72 hours of storage. Except for the difference in the onset of
324 equilibrium, samples packed with 70/30 (Figure 3) are a good representation to the trends seen for
325 samples packed with 50/50 and 35/65 (data not shown). As for all equilibrium reactions, the dissolution
326 of CO_2 is driven by the differences in CO_2 concentration within the fish and in the gas phase (Sivertsvik
327 *et al.*, 2004). This explains the slower uptake of CO_2 in the 35/65 samples. The majority of the samples
328 showed a constant level of estimated dissolved amount of CO_2 after 96 hours of storage. This shows
329 that equilibrium has been reached after 4 days of storage. For some samples, equilibrium is reached
330 even earlier. These findings are in agreement with the findings of Sivertsvik and Jensen (2005) and

Sivertsvik *et al.* (2004), who found MA packed fish and meat products reached equilibrium after 3 to 4 days of storage.

To the best of our knowledge, this is the first study to measure the concentration of dissolved CO_2 in a fish product as a function of time using different gaseous combination. However, Meredith et al. (2014) performed at storage study on the effect of MAP of poultry fillets on CO_2 concentration in the meat. They showed that samples with similar gaseous combination had an increase in amount of dissolved CO_2 for the first 2-4 days where after equilibrium was reached. This is in agreement with the findings in this study. At the end of the storage period, the CO_2 concentration in the chicken fillets reached levels similar to those observed in the present study.

Studies have showed that lipid composition of salmon muscle varies with feed type (organic or conventional) (Lerfall et al., 2016), breeding method (farmed or wild) as well as season (Hamilton et al., 2005), indicating that even seemingly similar products can be highly different when it comes to the use of MAP. Therefore, the results of this study concludes that solubility of CO_2 in a seafood model product with added lipids is highly dependent on storage temperature and MA gas composition. And more importantly, the solubility of CO_2 is correlated with the sum of liquid (water and liquid fat) showing that the solubility of CO_2 is more dependent on the state of- rather than the type of constituents. Lastly, this study showed that measurements of dissolved concentration of CO_2 is an unsuitable measure for a comparison of solubility between days, treatments and/or samples. Henry's constant gives a better basis for comparison. These findings explain why previous studies on the solubility of CO_2 have had highly contradicting results. Furthermore, the findings stress the need for the food industry to understand their products, as well as making individual adjustments in the use of MAP based on specific products and conditions in order to obtain the optimal condition for the shelf life prolongation for the foods.

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References

Bligh, E.G., Dyer, W.J., (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37(8), 911-917.

Caroll, J., Slupsky, J., Mather, A., (1991). The solubility of carbon-dioxide in water at low-pressure. Journal of Physical and Chemical Reference data 20(6), 1201-1209.

Devlieghere, F., Debevere, J., van Impe, J.F., (1998a). Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. International Journal of Food Microbiology 43(1), 105-113.

Devlieghere, F., Debevere, J., Van Impe, J.F., (1998b). Effect of dissolved carbon dioxide and temperature on the growth of *Lactobacillus sake* in modified atmospheres. International Journal of Food Microbiology 41(3), 231-238.

Fava, P., Piergiovanni, L., (1992). Carbon-dioxide solubility in foods packaged with modified atmosphere. 2: correlation with some chemical-physical characteristics and composition. Industrie Alimentari 31(304), 424-430.

Genigeorgis, C.A., (1985). Microbial and safety implications of the use of modified atmospheres to extend the storage life og fresh meat and fish. International Journal of Food Science and Technology 1(5), 237-251.

Gill, C.O., (1988). The solubility of carbon dioxide in meat. Meat Science 22(1), 65-71.

Gill, C.O., Penney, N., (1988). The effect of the initial gas volume to meat weight ratio on the storage life of chilled beef packaged under carbon dioxide. Meat Science 22(1), 53-63.

Gram, L., Huss, H.H., (1996). Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 33(1), 121-137.

Hamilton, M.C., Hites, R.A., Schwager, S.J., Foran, J.A., B.A., K., Carpenter, D.O., (2005). Lipid Composition and Contaminant in Farmed and Wild Salmon. Environmental Science & technology 39(22), 8622-8629.

Hansen, A.Å., Rødbotten, M., Lea, P., Rotabakk, B.T., (2015). Effect of transport packaging and repackaging into modified amtosphere on shelf life and quality of thawed Atlantic cod Loins. Packaging Technology and Science 28(11), 925-938.

Hellevik, A.H., Synnes, M., Stoknes, I., (2005). Underutilised fish species: Raw material properties of muscle fracton from Greater Argentine, Northern Wolffish and Polar Cod. Part of the project *Consumer products* in the EU-project SEAFOOD*plus*.

ISO.6496, (1983). Determination of Moisture and Other Volatile Matter Content. The International Orgaization for Standardization, Genf, Switzerland.

Jakobsen, M., Bertelsen, G., (2004). Predicting the amount of carbon dioxide absorbed in meat. Meat Science 68(4), 603-610.

Jakobsen, M., Bertelsen, G., (2006). Solubility of carbon dioxide in fat and muscle tissue. Journal of Muscle Foods 17(1), 9-19.

Jakobsen, M., Jensen, P.N., Risbo, J., (2009). Assessment of carbon dioxide solubility coefficients for semihard cheeses: the effect of temperature and fat content. European Food Research and Technology 229(2), 287-294.

Lambert, A.D., Smith, J.P., Dodds, K.L., (1991). Shelf life extension and microbiological safety of fresh meat - A review. Food Microbiology 8(4), 267-297.

Lerfall, J., Bendiksen, E.Å., Olsen, J.V., Morrice, D., Østerlie, M., (2016). A comparative study of organic- versus conventional farmed Atlantic salmon. I. Pigment and lipid content and composition, and carotenoid stability in oce-stored fillets. Aquaculture 451, 170-177.

McMurry, J., Simanek, E., (2007). *Fundamentals of Organic Chemistry*. Thomson Brooks/Cole, Belmont, USA.

Mendes, R., Gonçalves, A., (2008). Effect of Soluble CO2 Stabilization on the Qualityof Fillets From Farmed Gilthead Sea Bream (Sparus aurata) and European Sea Bass (Dicentrarchus labrax). Journal of Aquatic Food Produc Technology 17(4), 342-366.

412 Mendes, R., Silva, H.A., Anacleto, P., Cardoso, C., (2011). Effect of CO2 dissolution on the shelf life of
413 ready-to-eat Octopus vulgaris. Innovative Food Science & Emerging Technologies 12(4), 551-561.
414 Meredith, H., Valdramidis, V., Rotabakk, B.T., Sivertsvik, M., McDowell, D., Bolton, D.J., (2014). Effect
415 of different modified atmospheric packaging (MAP) gaseous combinations on Campylobacter and the
416 shelf-life of chilled poultry fillets. Food Microbiology 44, 196-203.
417 Metcalfe, L.D., Schmitz, A.A., Pelka, J.R., (1966). Rapid preparation of fatty acis esters from lipids for
418 gas chromatographic analysis. Analytical Chemistry 38, 514-515.
419 Powell, S.M., Tamplin, M.L., (2012). Microbial communities on Australian modified atmosphere
420 packaged Atlantic salmon. Food Microbiology 30(1), 226-232.
421 Rotabakk, B.T., (2013). Solubility of carbon dioxide in a seafood model product with fish oil. European
422 Food Research and Technology 237(4), 541-545.
423 Rotabakk, B.T., Birkeland, S., Lekang, O.I., Sivertsvik, M., (2008). Enhancement of Modified
424 Atmosphere Packaged Farmed Atlantic Halibut (*Hippoglossus Hippoglossus*) Fillet Quality by Soluble
425 Gas Stabilization. Food Science and Technology International 14(2), 179-186.
426 Rotabakk, B.T., Lekang, O.I., Sivertsvik, M., (2007). Volumetric method to determine carbon dioxide
427 solubility and absorption rate in foods packaged in flexible or semi rigid package. Journal of Food
428 Engineering 82(1), 43-50.
429 Rotabakk, B.T., Lekang, O.I., Sivertsvik, M., (2010). Solubility, absorption and desorption of carbon
430 dioxide in chicken breast fillets. Food Science and Technology 43(3), 442-446.
431 Rotabakk, B.T., Sivertsvik, M., (2012). Solubility of carbon dioxide in muscle foods and its use to
432 extend the shelf life of packaged products, in: Kerry, J.P. (Ed.), *Advances in Meat, Poultry and Seafood*
433 *packaging*. Elsevier, pp. 314-330.
434 Rumpf, B., Nicolaisen, H., Öcal, C., Maurer, G., (1994). Solubility of carbon-dioxide in aqeuos-
435 solutions of sodium-chloride: experimental results and correlation. Journal of Solution Chemistry
436 23(3), 431-448.
437 Schumpe, A., Quicker, G., Deckwer, W., (1982). Gas solubility in microbial culture media. Journal of
438 Solution Chemistry 24(6), 405-421.
439 Sivertsvik, M., Jeksrud, W.K., Rosnes, J.T., (2002). A review of modified atmosphere packaging of fish
440 and fishery products - significance of microbial growth, activities and safety. International Journal of
441 Food Science and Technology 37(2), 107-127.
442 Sivertsvik, M., Jensen, J.S., (2005). Solubility and absorption rate of carbon dioxide into non-respiring
443 foods. Part 3: Cooked meat products. Journal of Food Engineering 70(4), 499-505.
444 Sivertsvik, M., Rosnes, J.T., Jeksrud, W.K., (2004). Solubility and absorption rate of carbon dioxide
445 into non-respiring foods. Part 2: Raw fish fillets. Journal of Food Engineering 63(4), 451-458.
446 Sivertsvik, M., Rosnes, J.T., Kleiberg, G.H., (2003). Effect of Modified Atmosphere Packaging and
447 Superchilled Storage on the Microbial and Sensory Quality of Atlantic Salmon (Salmo salar) Fillets.
448 Journal of Food Science 68(4), 1467-1472.
449 Speranza, B., Corbo, M.R., Conte, A., Sinigaglia, M., Del Nobile, M.A., (2009). Microbiological and
450 Sensorial Quality Assessment of Ready-to-Cook Seafood Products Packaged under Modified
451 Atmosphere. Journal of Food Science 74(9), 473-478.
452 Torrieri, E., Cavella, S., Villani, F., Masi, P., (2006). Influence of modified atmosphere packaging on the
453 chilled shelf life of gutted farmed bass (Dicentrarchus labrax). Journal of Food Engineering 77(4),
454 1078-1086.
455 Tsironi, T.N., Taukis, P.S., (2010). Modeling Microbial Spoilage and Quality of Gilthead Seabream
456 Fillets: Combined Effect of Osmotic Pretreatment, Modified Atmosphere Packaging, and Nisin of
457 Shelf Life. Journal of Food Science 75(4), 243-251.
458 Özogul, F., Polat, A., Özogul, Y., (2004). The effects of modified atmosphere packaging and vacuum
459 packaging on chemical, sensory and microbiological changes of sardines (Sardina pilchardus). Food
460 Chemistry 85(1), 49-57.

461

Table 1 Lipid content, water content, and fatty acid composition of the eight different model products. Only fatty acids composing more than 5% of the total lipid content are included.

Sample	Lipid [%] (n=8)	Water [%] (n=8)	Sum lipid and water [%]	Sum water and liquid lipid [%]	Fatty acid main constituents
Recipe 1	18.8±1.0^{a}	64.6±0.4^{b}	83.5±1.1^{a}	64.6±0.4^{d,e}	C18:0 (60%) C18:1 (37%)
Recipe 2	19.8±1.6^{a}	63.8±0.8^{b}	83.8±1.3^{a}	73.8±0.8^{b}	C18:0 (57%) C20:5 (23%) C22:6 (15%)
Recipe 3	18.9±0.8^{a}	64.2±0.5^{b}	82.9±0.9^{a}	73.5±0.6^{b}	C18:1 (54%) C20:5 (21%) C22:6 (14%)
Recipe 4	19.4±0.5^{a}	64.0±0.2^{b}	83.3±0.5^{a}	70.4±0.2^{c}	C18:0 (36%) C18:1 (34%) C20:5 (14%) C22:6 (10%)
Recipe 5	18.5±0.7^{a}	64.6±0.5^{b}	83.2±1.1^{a}	64.6±0.5^{d}	C18:0 (99%)
Recipe 6	18.5±0.2^{a}	63.9±0.7^{b}	82.4±0.8^{a}	63.9±0.7^{e}	C18:1 (93%)
Recipe 7	14.5±0.5^{b}	64.1±0.5^{b}	78.3±0.6^{b}	78.3±0.6^{a}	C20:5 (50%) C22:6 (37%)
Recipe 8	0.6±0.4^{c}	78.1±0.3^{a}	78.9±0.3^{b}	78.1±0.3^{a}	C16:0 (32%) C18:1 (17%) C20:1 (12%) C22:6 (15%)

Different superscript (a,b,c,d,e) in each column indicate significant variation ($p<0.05$) by one-way ANOVA and Tukey's pairwise comparison test.

Recipe 1: 50/50 oleic/stearic acid, recipe 2: 50/50 Stearic/DHA and EPA mix, recipe 3: 50/50 Oleic/DHA and EPA mix, recipe 4: 33/33/33 Oleic/Stearic/DHA and EPA mix, recipe 5: 100% stearic acid, recipe 6: 100% oleic acid, recipe 7: 100% DHA and EPA mix, and recipe 8: control.

Table 2 Main effect of packaging gas composition, fatty acid profile, and storage temperature on equilibrium headspace gas composition, equilibrium concentration of dissolved CO_2 in the model product, and Henry's constant adjusted for content of water, content of water and lipid, and content of water and liquid lipid, respectively. Key results are elaborated in Figure 3 and 4

	Headspace CO_2 [%]	**Equilibrium CO_2 concentration [ppm]**	**Henry's constant[1] [Pa ppm^{-1}]**	**Henry's constant[2] [Pa ppm^{-1}]**	**Henry's constant[3] [Pa ppm^{-1}]**
35	25±3	706±140	57±17	46±15	51±159
50	32±39	954±166	53±14	43±11	48±12
70	45±4	1376±235	52±13	41±9	48±16
Recipe 1	35±9	899±260	63±14	48 ±11	60±10
Recipe 2	33±9	1020±314	51±10	39±8	45±9
Recipe 3	32±9	1013±316	52±10	40±8	43±6
Recipe 4	33±9	991±324	55±11	42±9	48±8
Recipe 5	34±9	964±295	56±11	43±9	56±11
Recipe 6	35±9	940±292	61±11	47±9	55±5
Recipe 7	34±9	1153±388	50±21	41±18	41±18
Recipe 8	35±9	1140±395	43±18	44±18	46±25
0	31±8	1182±339	41.4±6.1	33.0±4	39±8
8	34±9	1030±291	50.9±7.5	40.5±4	48±9
20	37±9	825±264	70.2±12.0	56.0±11	61±15

[1] Adjusted for water content
[2] Adjusted for water- and total lipid content
[3] Adjusted for water- and liquid lipid content
Recipe 1: 50/50 oleic/stearic acid, recipe 2: 50/50 Stearic/DHA and EPA mix, recipe 3: 50/50 Oleic/DHA and EPA mix, recipe 4: 33/33/33 Oleic/Stearic/DHA and EPA mix, recipe 5: 100% stearic acid, recipe 6: 100% oleic acid, recipe 7: 100% DHA and EPA mix, and recipe 8: control.

Figure captions

Figure 1: Composition of lipid mixtures added to the fish model product recipe 1 through 7. All mixtures were added to a total of 18% added lipid in the final product. A control, recipe 8, without addition of external lipids was included in the experiment. Recipe 1: 50/50 oleic/stearic acid, recipe 2: 50/50 Stearic/DHA and EPA mix, recipe 3: 50/50 Oleic/DHA and EPA mix, recipe 4: 33/33/33 Oleic/Stearic/DHA and EPA mix, recipe 5: 100% stearic acid, recipe 6: 100% oleic acid, recipe 7: 100% DHA and EPA mix, and recipe 8: control.

Figure 2: DSC results for phase transition temperatures of pure oleic acid (A), pure stearic acid (B), and recipe 5 containing the addition of 100% pure stearic acid (C) and recipe 6 containing the addition of 100% oleic acid (D), regardless of transition energy.

Figure 3: Concentration of CO_2 [ppm] in samples recipe 1 through 8 packed with 70% CO_2 in packaging gas and stored at 0, 8, and 20˚C. Error bars indicates SD. . Recipe 1: 50/50 oleic/stearic acid, recipe 2: 50/50 Stearic/DHA and EPA mix, recipe 3: 50/50 Oleic/DHA and EPA mix, recipe 4: 33/33/33 Oleic/Stearic/DHA and EPA mix, recipe 5: 100% stearic acid, recipe 6: 100% oleic acid, recipe 7: 100% DHA and EPA mix, and recipe 8: control.

*Figure 4: Henry's constant adjusted for water- and liquid lipid content for samples recipe 1 through 8 stored at 0°, 8°, and 20°C. Error bars indicates SD. * most of the recipe 8 (control) samples stored at 20°C collapsed during the storage period, leading to highly irregular results, thus error bars has been removed, however the column is included to indicate the mean value. Recipe 1: 50/50 oleic/stearic acid, recipe 2: 50/50 Stearic/DHA and EPA mix, recipe 3: 50/50 Oleic/DHA and EPA mix, recipe 4: 33/33/33 Oleic/Stearic/DHA and EPA mix, recipe 5: 100% stearic acid, recipe 6: 100% oleic acid, recipe 7: 100% DHA and EPA mix, and recipe 8: control.*

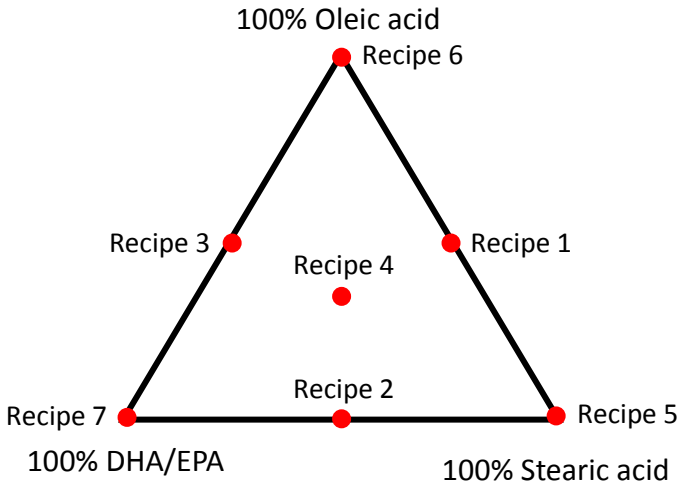
100% Oleic acid
Recipe 6
Recipe 3
Recipe 1
Recipe 4
Recipe 2
Recipe 7
Recipe 5
100% DHA/EPA
100% Stearic acid

Figure 1.

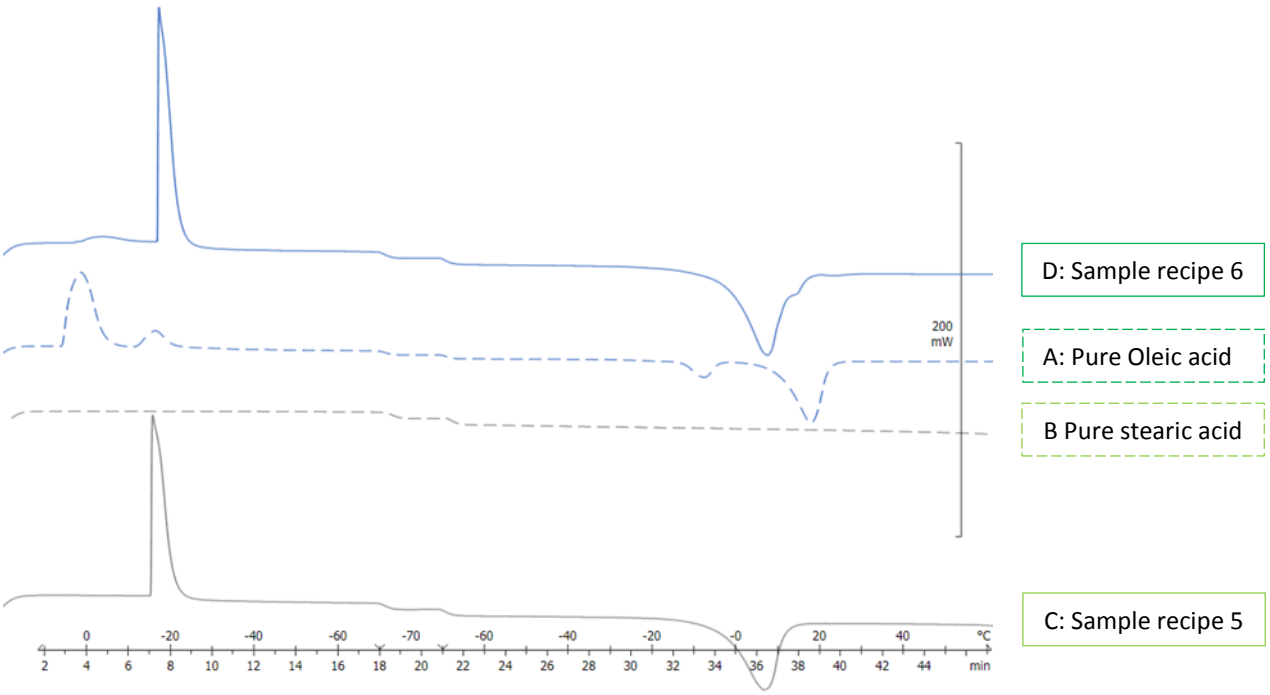
200
mW
D: Sample recipe 6
A: Pure Oleic acid
B Pure stearic acid
C: Sample recipe 5
0
-20
-40
-60
-70
-60
-40
-20
-0
20
40
°C
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
min

Figure 2.

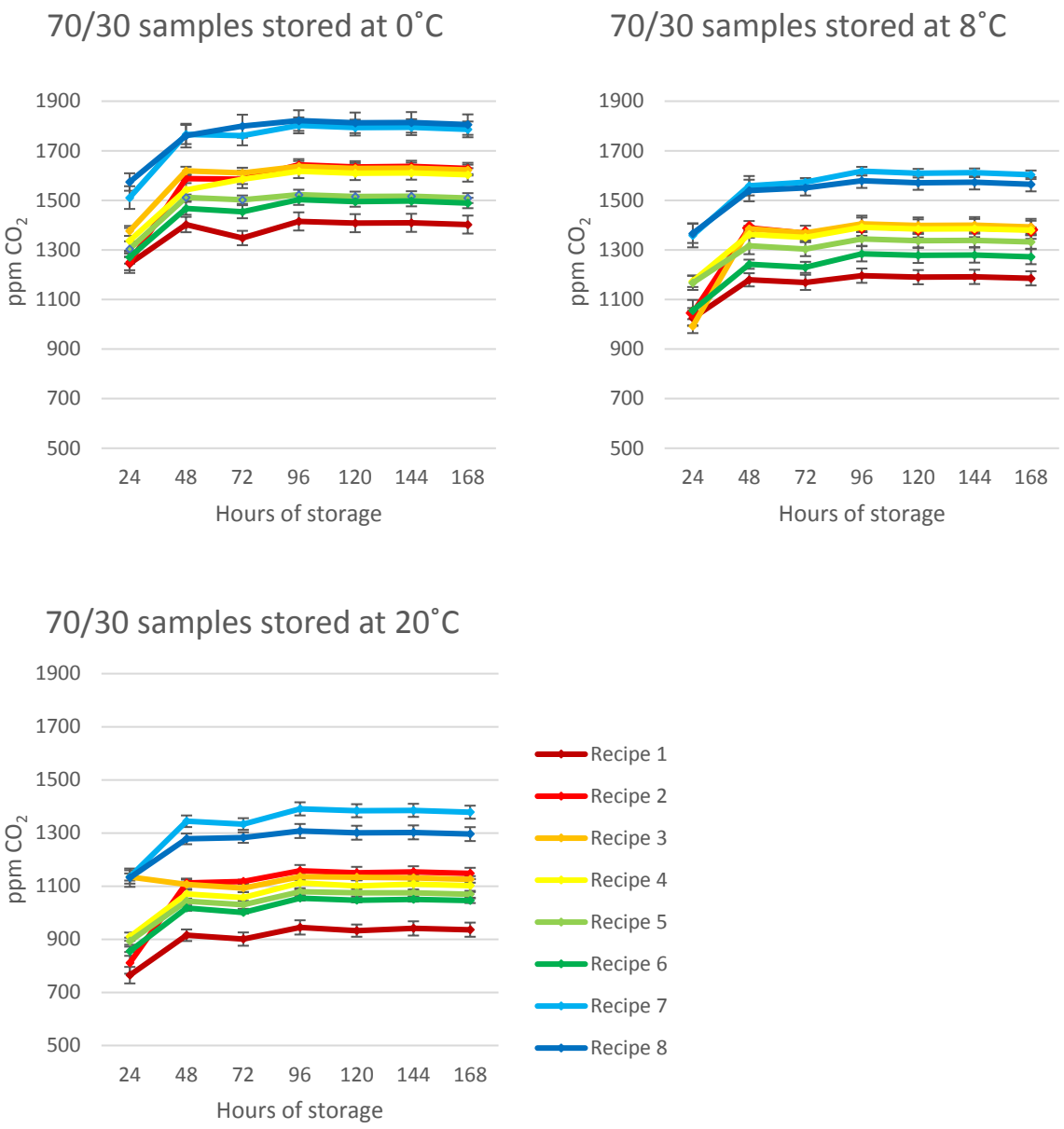
70/30 samples stored at 0˚C
70/30 samples stored at 8˚C
70/30 samples stored at 20˚C
ppm CO2
1900
1700
1500
1300
1100
900
700
500
24
48
72
96
120
144
168
Hours of storage
Recipe 1
Recipe 2
Recipe 3
Recipe 4
Recipe 5
Recipe 6
Recipe 7
Recipe 8

Figure 3.

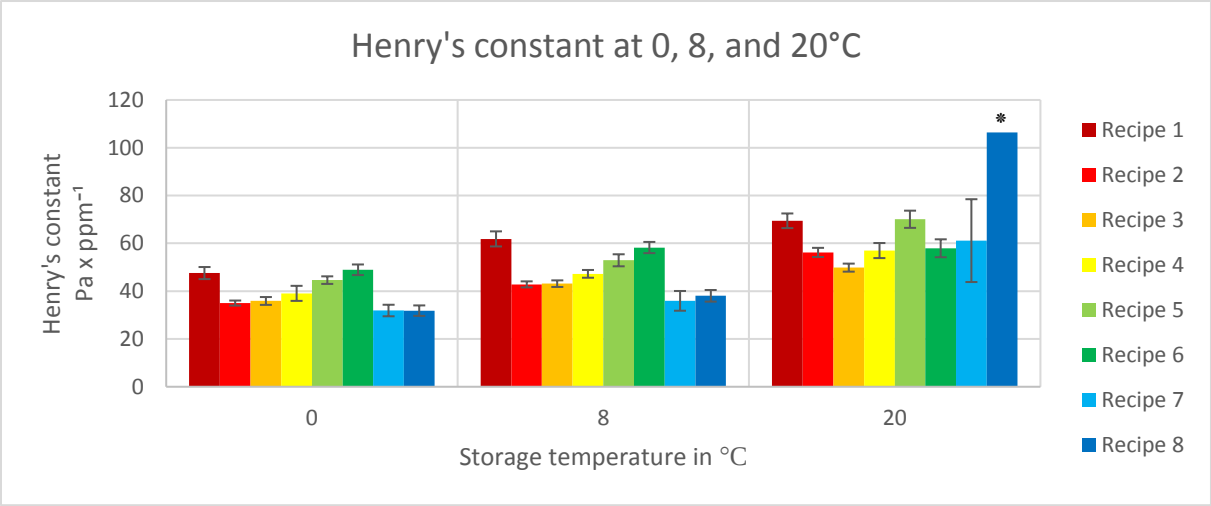
Henry's constant at 0, 8, and 20°C
Henry's constant
Pa x ppm⁻¹
120
100
80
60
40
20
0
0
8
20
*
Storage temperature in °C
Recipe 1
Recipe 2
Recipe 3
Recipe 4
Recipe 5
Recipe 6
Recipe 7
Recipe 8

Figure 4.