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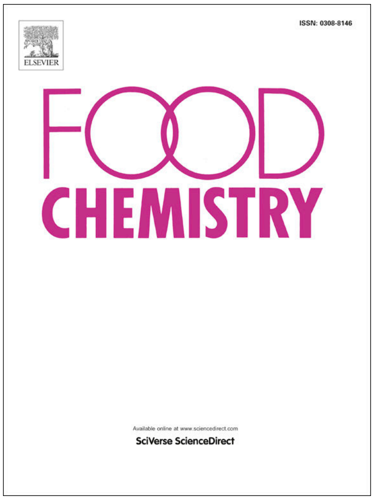

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Solubility of carbon dioxide in renneted casein matrices: Effect of pH, salt, temperature, partial pressure, and moisture to protein ratio

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Abstract

The solubility of carbon dioxide (CO_2) in the moisture and protein components of cheese matrices and the influence of changing pH, salt and temperature levels remains unclear. In this study, model casein matrices were prepared, by renneting of micellar casein concentrate (MCC), with modulation of salt and pH levels by adding salt and glucono delta-lactone, respectively to the MCC solutions prior to renneting. Different moisture-to-protein levels were achieved by freeze-drying, incubation of samples at different relative humidities, or by applying varying pressures during gel manufacture. The CO_2 solubility of samples decreased linearly with both increasing temperature and salt-in-moisture content, whereas solubility of CO_2 increased with increasing pH. A non-linear relationship was observed between CO_2 solubility and the moisture-to-protein ratio of experimental samples. Overall, such knowledge may be applied to improve the quality and consistency of eye-type cheese, and in particular to avoid development of undesirable slits and cracks.

Keywords: Carbon dioxide solubility; Casein matrices; Slit and crack defects; Eye-development

1. Introduction

In some eye-type cheeses, such as Emmental and Maasdam, propionic acid bacteria produce a high level of carbon dioxide, especially during warm-room ripening. The rate and extent of gas production and its behaviour in the cheese matrix (e.g., solubility and diffusivity) are considered important factors for the development of eyes, but are also implicated in the undesired development of slits or cracks within those cheese types (Daly, McSweeney, & Sheehan, 2010b; P. Lamichhane, 2019). It is believed that CO_2 produced within the cheese matrix first solubilizes/dissolves within the components of the cheese matrix. Once the cheese body becomes saturated with gas, it then diffuses to the nuclei for eye-development or diffuses outward through the cheese rind. It has been reported that ~50% of the total CO_2 gas produced is dissolved in the cheese body (Walstra, Wouters, & Geurts, 2005).

Studies have suggested that the CO_2 solubility within the cheese matrix largely depends on factors such as cheese composition, temperature, and partial pressure. Carbon dioxide solubilizes in both the aqueous and fat phases of cheese; however, the CO_2 solubility capacity of each phase is temperature-dependent (Jakobsen, Jensen, & Risbo, 2009). CO_2 solubility in the aqueous phase of cheese has been reported to decrease with increasing temperature whereas the CO_2 solubility in the fat phase has been reported to increase with increasing temperature (Jakobsen et al., 2009). Acerbi, Guillard, Guillaume, and Gontard (2016) studied the effect of temperature, partial pressure, salt and moisture content on the solubility behaviour of CO_2 in semi-hard cheese. Those authors observed a decrease in CO_2 solubility with increasing temperature and salt level. However, a complex relationship was observed with moisture level, which has been attributed to concomitant changes in protein content with changing moisture levels. Those authors recommended conducting further research to clarify the influence of nitrogen content on CO_2 solubility in cheese.

Although several studies have investigated CO_2 solubility behaviour in food matrices (Acerbi et al., 2016; Adhikari, Truong, Bansal, & Bhandari, 2018) or in pure fat (Jakobsen et al., 2009; Truong, Palmer, Bansal, & Bhandari, 2017), the solubility behaviour of CO_2 in dairy protein matrices is not yet fully understood. In fact, studies have neglected the effect of protein content on CO_2 solubility (Acerbi et al., 2016; Jakobsen et al., 2009). However, it is difficult to investigate the effect of each individual component on solubility behaviour in a multi-component food system, as changing of one compositional parameter results in consequential changes to other compositional parameters. Therefore, studies using model systems may be better suited to understand the effect of each component individually on solubility behaviour of CO_2.

The primary aim of this study was to investigate the effect of moisture-to-protein ratio on the solubility of CO_2 in renneted-casein gel matrices rather than simply in a protein-only matrix, as it is not possible to vary protein content without changing the moisture level. In the majority of food matrices, protein is mostly present in a hydrated state, and its level of hydration is dependent on product type; for example, there are low levels of protein hydration in dairy powders compared to cheese. An additional aim of this study was to elucidate the effect of varying levels of salt, pH, temperature and partial pressure on the solubility of CO_2 in model renneted-casein gel matrices.

2. Materials and methods

2.1. Preparation of renneted casein matrix

Liquid micellar casein concentrate (MCC; protein content = 14.55%, w/w; total solids: 18.34%, w/w) was produced as reported by Xia et al. (accepted for publication, International Dairy Journal, article number: 104796) and stored at -18 °C. Prior to use in experiments, the MCC was thawed in a water bath at 50 °C and an aliquot (400 g) was placed in a 500 mL beaker with 0.03 % (w/w) sodium azide (BDH Chemicals, Poole, England) as a preservative. The desired salt concentration and pH levels of the final gels, were achieved by mixing varying levels of NaCl (0, 1.5, or 2.5%, w/w) and glucono-δ-lactone (GDL; 0.5, 1.2, or 2%, w/w; Sigma-Aldrich) into the MCC with a magnetic stirrer. Three minutes after salt and GDL addition, fermentation-produced bovine chymosin (FPBC; CHY-MAX Plus, ~200 international milk clotting units (IMCU)/mL; Chr. Hansen Ltd., Cork, Ireland) was added at a level of 0.82 mL kg^{-1} MCC. Rennet addition was based on MCC protein content. All renneted milk concentrates were incubated at 32 °C for 30 min to induce gel formation and stored overnight at 4 °C for completion of GDL hydrolysis.

On the following day, all gels were incubated in a water bath at 40 °C for 1 h to promote expulsion of whey/moisture. Each gel was then collected into a mould and pressed vertically under increasing pressure (up to 195 kPa) for 3 h to obtain the desired final moisture content. All gels were then vacuum-packed (Falcon 52, Original Henkelman vacuum system, the Netherlands), and stored at 4 °C.

2.1.1 Preparation of a model system to investigate the effect of partial pressure and temperature

To investigate the effect of partial pressure and temperature on CO_2 solubility, three identical casein matrices were prepared of moisture and salt content ~60% (w/w) and ~2% (w/w), respectively, and with a pH of ~5.8.

2.1.2 Preparation of a model system to investigate the effect of salt

To investigate the effect of salt on the CO_2 solubility, three casein matrices were prepared in triplicate, by adding three different salt levels, i.e., 0, 1.5, and 2.5%, w/w, to the MCC. Other parameters, including levels of protein and moisture, and pH, were all kept constant.

2.1.3 Preparation of a model system to investigate the effect of pH

To investigate the effect of pH on the CO_2 solubility, three casein matrices, of three different pHs, were prepared in triplicate, by adding varying levels of GDL (0.5, 1.2, or 2%, w/w) to the MCC. Other parameters, including levels of salt, protein and moisture, were all kept constant. A series of preliminary experiments were initially conducted to determine the levels of GDL necessary to achieve the desired pH value, which ranged between 5.4 and 6.2.

2.1.4 Preparation of a model system to investigate the effect of moisture-to-protein ratio

Approaches, such as application of variable pressure during manufacture, freeze-drying, or incubation of samples in various relative humidity environments, were applied to achieve desired hydration levels (moisture-to-protein ratio) of the casein matrices. Increasing pressing pressure up to 98 kPa for 2 h was applied to achieve casein matrices with a moisture content of ~67% (w/w), whereas increasing pressure up to 197 kPa for 3 h was applied to achieve casein matrices with a moisture content of ~59% (w/w). Casein matrices with a moisture content of ~47% (w/w) or ~34% (w/w) were prepared by incubating small slices of casein gel (each of ~2 g, initial moisture content of ~59%) for 1 to 2 weeks, at 4 °C, in desiccators containing a saturated solution of LiCl. Casein matrices of very low moisture content (~2%, w/w) were prepared by freeze drying. Some freeze-dried samples were rehydrated to achieve a moisture content of 15% (w/w) or 19% (w/w) by incubating (for 2 weeks at 25 °C) in a hermetically sealed container maintaining a relative humidity of 97% (using saturated potassium sulphate) or 100% (using pure water), respectively.

2.2. Composition analysis

Moisture, protein and salt contents were determined as described by P. Lamichhane, Kelly, and Sheehan (2018a). The fat content of samples was determined using the Röse-

Gottlieb method (IDF, 1996). The pH of gel samples was determined by directly inserting a penetrating pH probe (HQ11d, Hach, Cork, Ireland) into gel samples.

2.3. Determination of freezable and non-freezable moisture

Levels of freezable and bound moisture were determined using a differential scanning calorimeter (DSC; Q200, TA Instruments, New Castle, DE, USA) as described by McMahon, Fife, and Oberg (1999). Freezable moisture is defined as water freezeable at −40 °C, whereas non-freezable moisture was defined as the water that did not freeze at −40 °C (McMahon et al., 1999).

2.4. Modified atmosphere packaging

In triplicate, experimental samples of 1.5 to 2 g, were each individually packaged into pouches (length = 25 cm, width = 18.5 cm; Amcor Flexibles, Denmark) of high gas impermeability using a modified atmosphere packaging machine equipped with a two-gas mixture (A300; Multivac, Germany). To investigate the effect of partial pressure and temperature on CO_2 solubility, samples were packed with a CO_2:N_2 gas mixture of 0:100; 30:70; 60:40 and 100:0 and stored at 5 °C, 12 °C and 25 °C for 2 d. To investigate the effect of salt, pH and casein hydration, samples were packed under 100% CO_2 and stored at 5 °C for 2 d.

2.5. Determination of the concentration of CO_2 in the headspace of modified atmosphere packages

The concentration of CO_2 in the headspace of modified atmosphere packages was determined using a headspace gas analyser (CheckMate 9900, PBI-Dansensor A/S, Ringsted, Denmark). To prevent gas leakage from the packaging material during measurement, a septum (diameter = 15 mm, MACON Europe A/S, Denmark) was attached to the top of the packaging materials which was pierced by the needle of the headspace gas analyser.

2.6. Determination of the concentration of CO_2 in renneted-casein matrices

The concentration of carbon dioxide in the renneted-casein matrix was determined using a titration method as described in previous studies (Gill, 1988; Jakobsen et al., 2009; Truong et al., 2017). Briefly, a pair of side-armed conical flasks (100 mL; Pyrex), one containing 10 mL of 0.5 M H_2SO_4 and another containing 3 mL of 0.1 N $Ba(OH)_2$, connected by a reinforced PVC tube, were used for extraction and subsequent scavenging of CO_2 from experimental samples. Experimental samples (~1.5 g) held under modified atmosphere packaging were transferred immediately to the flask containing 0.5 M H_2SO_4, which was sealed using a neoprene stopper. Therein, the CO_2 released from the experimental sample reacted with $Ba(OH)_2$ (present in the other flask) and produced a $BaCO_3$ precipitate. After at least 24 h, the residual $Ba(OH)_2$ was titrated against a standard HCl solution (0.1 M) using phenolphthalein as an indicator.

2.7. Statistical analysis

Statistical analyses of the data were performed using SigmaPlot version 14 (Systat Software, Inc., San Jose, California, USA). The effect of treatment on CO_2 solubility of casein matrices was determined performing one way ANOVA followed by *post-hoc* Student-Newman-Keuls tests. Before ANOVA evaluation, data were checked for homoscedasticity and normality by performing Brown-Forsythe and Shapiro–Wilk tests, respectively. The level of significance was set at $P \leq 0.05$. Regression analyses of the data were performed using SigmaPlot version 14 (Systat Software, Inc., San Jose, California, USA).

3. Results and discussions

3.1. Effects of partial pressure and temperature on solubility of CO_2

A linear relationship was observed between the concentrations of CO_2 in the experimental samples and the CO_2 partial pressure of the headspace of corresponding samples at all three temperatures investigated (Fig. 1a). These results are in agreement with previous studies on cheese (Acerbi et al., 2016; Jakobsen et al., 2009) and on anhydrous milk fat (Truong et al., 2017). The linear regression equations obtained had very high coefficients of determination (R^2 = 0.98-0.99), thus validating Henry's law for the casein matrix studied. However, a small deviation from the origin was observed at zero partial pressure of CO_2. This deviation in CO_2 solubility of samples ranged between 0 and 2.68 mmol kg^{-1}. Similar deviations from the origin have previously been observed in cheese (Acerbi et al., 2016; Jakobsen et al., 2009) and in anhydrous milk fat (Truong et al., 2017) and were attributed to the inherent presence of carbamate or carbonate species within the sample (Acerbi et al., 2016; Jakobsen et al., 2009). We believe that such a small offset plays a negligible role in the overall determination of the relationship between the physicochemical parameters of the matrix and CO_2 solubility.

The solubility of CO_2 in a casein matrix decreased ($P < 0.05$) linearly ($R^2 = 0.96$) as the temperature increased, with ~35% lower CO_2 solubility observed at 25 °C than at 5 °C (Fig. 1b). It is proposed that the random molecular motion of the CO_2 gas molecules increases with increasing temperature (Cofie-Agblor, Muir, Sinicio, Cenkowski, & Jayas, 1995), thereby reducing the forces of attraction between CO_2 gas and the casein matrix, with subsequent release of CO_2 from the casein matrix.

Slits and cracks are usually observed during cold room storage and, therefore, we propose that the changes in CO_2 solubility with changing ripening temperature may contribute to the occurrence of such defects. Cheeses such as Maasdam and Emmental are pre-ripened for 1 to 2 weeks at 8-10 °C before warm room ripening (~23 °C) for 4 to 6 weeks for the development of eyes, and are finally stored at 2-4 °C. Propionic acid bacteria produce a high level of CO_2, especially during warm-room ripening, and a proportion (~50%) of CO_2 produced dissolves in the cheese body, while the remainder diffuses to nuclei for eye-formation (~20%) and diffuses outward (~30%) through the cheese rind. During ripening, the cheese texture become more brittle (i.e., fracturing of cheese matrix at a relatively small deformation), most probably due to solubilisation of colloidal calcium, hydrolysis of caseins, or both (P. Lamichhane, Sharma, Kennedy, Kelly, & Sheehan, 2019). Moreover, the cheese texture also becomes brittle during cold storage due to solidification of fat. This suggests that sudden and significant increase in cheese storage temperature due to a range of circumstances, such as increased external ambient temperature or the arrival of new batches of cheese (from warm rooms) into the cold rooms decrease the gas solubility and this increases the CO_2 partial pressure within the cheese matrix. Such unintended increases in partial pressure at later stages of ripening (i.e., during cold storage temperature) may overcome the adhesive or cohesive strength of the cheese matrix, leading to initiation and propagation of cracks and slits within the cheese matrix (Prabin Lamichhane, Auty, Kelly, & Sheehan, 2020). Overall, this suggests that strict control of cheese storage temperature may contribute to minimizing and avoidance of development of slit and crack defects.

3.2. Effect of salt content on solubility of CO_2

The effect of three different salt concentrations on CO_2 solubility within the casein matrices was studied (Fig. 2). Carbon dioxide solubility in the casein matrices decreased (P = 0.004) by ~22% with increasing average salt content from 0.04% (salt-in-moisture content: 0.06%, w/w) to 1.89% (salt-in-moisture content: 3.13%, w/w). This decrease in solubility may be attributed to a salting-out effect on the solubility of CO_2 in the aqueous phase of the casein matrix by NaCl, as reported in previous studies (Acerbi et al., 2016; Carvalho, Pereira, Gonçalves, Queimada, & Coutinho, 2015). In other words, salt is more polar than CO_2 and, in solution, polar water molecules are more attracted to polar salt ions than nonpolar CO_2 molecules. Thus, fewer water molecules are available for dissolution of CO_2 in salt solutions, resulting in reduced solubility of CO_2 (Holzammer, Finckenstein, Will, & Braeuer, 2016). Moreover, increasing the salt concentration of aqueous solutions increases interfacial tension providing another reason for a decrease in CO_2 solubility with increasing salt concentration (Bachu & Bennion, 2009). Although the effect of salt on solubility behaviour of CO_2 in aqueous solutions is well known, the role of NaCl on CO_2 solubility behaviour in solid food matrices is less documented. Acerbi et al. (2016) studied the impact of salt content on CO_2 solubility behaviour in semi-hard cheese matrices and, in agreement with our results, observed a significant decrease in CO_2 solubility (by ~25%) on increasing salt levels from 0.0 to 2.7 % (w/w).

The salt (or salt-in-moisture) concentration range selected was similar to those found in eye-type cheeses and those involving propionic acid bacteria (PAB) fermentation cheeses (Guinee, 2004; P. Lamichhane et al., 2019). In brine-salted cheese types, salt diffuses inward from the rind to the centre of the cheese matrices, resulting in a decreasing salt gradient from the rind to the centre (Guinee, 2004). The time for attainment of equilibrium in salt-in-moisture content within the cheese matrix depends on composition, size and shape of the cheese and ripening conditions, among other factors. It has been reported that Gouda (10 kg wheel) and Emmental (60-130 kg wheel) cheese takes 7-9 weeks and > 4 months, respectively, for attainment of equilibrium in salt-in-moisture content within the cheese matrix (Daly, McSweeney, & Sheehan, 2010a; Guinee, 2004). Salt content in cheese can vary from batch to batch due to manufacture-derived variables (e.g., temperature of brine and brining time), or over a season due to variation in the composition of milk. Daly et al. (2010a) observed a significantly higher salt content in the interior area of Emmental cheese blocks produced late in the season of manufacture than those produced early in the season. Such intra-cheese and inter-cheese variation in salt content will result in heterogeneity in the local concentration of CO_2 within or between cheese blocks, leading to variable internal pressure within or between cheeses. This may result in some areas of the cheese matrices or some batches of cheeses being more prone to the development of slits or cracks. Although no specific locations for development of slits and cracks within cheese matrices have been reported, a lower number of eyes have been found near the rind of Emmental cheese, where salt content is higher, than in the centre of the cheese (Bisig et al., 2019).

3.3. Effect of pH on solubility of CO_2

Solubility of CO_2 within the casein matrices increased ($P < 0.001$) by ~41% on increasing pH from 5.4 to 6.15 (Fig. 3) with a quadratic relationship ($R^2 = 0.93$) providing a better fit than a linear relationship ($R^2 = 0.84$). Interestingly, the solubility of CO_2 increased ($P = 0.026$) by ~14% on increasing pH from 5.4 to 5.8, whereas the solubility of CO_2 increased ($P = 0.001$) by ~24% on increasing pH from 5.4 to 6.2. Such an increase in solubility is attributed to dissociation of an increasing fraction of the dissolved CO_2 as HCO_3^- with increasing pH (Gill, 1988). In solution, CO_2 can exist as dissolved CO_2, carbonic acid, bicarbonate or carbonate ions, and the fraction of each form in solution depends on the pH of that solution (Equation 1). With increasing pH from 5.4 to 6.15, increasing quantities of carbonic acid will dissociate as bicarbonate ions (HCO_3^-) and hydrogen ions (H^+). As a result, higher quantities of CO_2 will dissolve in the aqueous phase of the casein matrix (Jakobsen & Bertelsen, 2002). At pH values below 8, the carbonate ions (CO_3^-) in solution are present in negligible amounts (Dixon & Kell, 1989).

$$H_2O + CO_2 \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+ \longleftrightarrow CO_3^- + 2H^+ \quad (1)$$

Very little is known regarding the effect of pH on CO_2 solubility in solid food systems and no consensus have been found among the studies. Gill (1988), on investigating the effect of pH of the muscle tissue of beef, pork and lamb, observed a linear increase in the solubility of CO_2 with increasing pH from 5.4 to 6.9. Similarly, Jakobsen and Bertelsen (2006) reported a slightly higher solubility of CO_2 in meat tissue with higher pH (5.83) than lower pH (5.66). However, a difference of 0.5 pH units in the two meat types or fish types did not influence the solubility of CO_2 (Sivertsvik & Jensen, 2005; Sivertsvik, Rosnes, & Jeksrud, 2004). This discrepancy may be attributed to a comparison of data between different samples with different compositions and possibly of different buffering capacity.

Dissolution of CO_2 in the aqueous phase of food matrices can decrease pH because of formation of carbonic acid (Singh, Wani, Karim, & Langowski, 2012). There was a concern that addition of CO_2 to the protein matrices would have resulted in a reduction in pH of the samples, thus confounding our results relating to the effect of pH on CO_2 solubility. Therefore, the pH of the samples was measured before and after packaging in a modified atmosphere. It was observed that storage of small pieces (~10 g) of the casein matrices (3.1 % salt-in-moisture, 60% moisture content, and 32% protein content) under a 100% CO_2 environment for 2 d reduced the pH by ~0.1 unit (data not shown). However, for water samples of similar initial pH and salt-in-moisture content, pH decreased by ~2.3 units when stored under 100% CO_2 environment for 2 d (data not shown). The comparatively small decrease in pH of the casein matrices as compared to water may be attributed to its high buffering capacity, as food matrices of higher buffering capacity are expected to exhibit a greater resistance to pH change. Buffering capacity of food matrices largely depends on their composition. Proteins, inorganic phosphate and organic acids are the main constitutes contributing to the buffering capacity of cheese (Salaün, Mietton, & Gaucheron, 2005).

During ripening, pH within the eye-type cheese matrices increases, due to the proteolytic liberation of basic compounds and metabolism of lactic acid by propionic acid bacteria, among other factors (P. Lamichhane, Kelly, et al., 2018a; Sheehan, Fenelon, Wilkinson, & McSweeney, 2007). Studies have reported an increase in pH from 5.2-5.3 at 7-11 d (before warm-room ripening) to ~5.5 at 35-41 d (after warm-room ripening) and 6.0-6.1 at 270 d of ripening in Swiss, Dutch and related eye-type cheeses (Govindasamy-Lucey, Jaeggi, Martinelli, Johnson, & Lucey, 2011; P. Lamichhane, Kelly, et al., 2018a). Therefore, an increase in pH, especially above pH 5.8, during ripening of cheese may contribute to an increase in solubility of CO_2 within the cheese matrices. Moreover, natural cheese matrices can have both macroscopic and microscopic pH gradients (Burdikova et al., 2015), which may lead to heterogeneity in the concentration of CO_2 within the cheese matrices.

3.4. Effect of moisture-to-protein ratio on CO_2 solubility

To investigate the effect of moisture-to-protein ratio on CO_2 solubility, casein matrices having different moisture and protein contents were prepared. Using approaches, such as application of variable pressure during manufacture, freeze-drying, or incubation of samples in various relative humidity environments, it was possible to obtain casein matrices with moisture-to-protein ratios ranging between 0.03 and 2.5 (or moisture content range between 2% and 67%).

Casein matrices with an average moisture-to-protein ratio of 0.027±0.009 [corresponding to an average moisture and protein content of 2.10±0.67% (w/w) and 78.72±1.26% (w/w) respectively] retained a considerable amount of CO_2, i.e., 161.7±24.68 mmol kg^{-1} atm^{-1} at 5 °C (Fig. 4), in agreement with the results of Mitsuda, Kawa, Yamamoto, and Nakajima (1975). Those authors observed that dried casein and gelatine powders retained a considerable amount of CO_2 when stored under high CO_2 partial pressure. Although the exact causes are not known, CO_2 adsorption by reactive sites in protein is considered as an important factor (Cundari et al., 2009; Mitsuda et al., 1977). Polar and charged residues of amino acids, such as ε-amino of lysine and guanidinium groups of arginine, are the potential reactive sites in protein for adsorption of CO_2. Mitsuda et al. (1977) investigated the reactivity of certain particular functional groups involved in CO_2 gas adsorption by protein. Those authors obtained a good correlation between the amount of CO_2 adsorbed and the total lysine and arginine content of protein samples and they concluded that the α-amino, ε-amino and guanidinium groups are the preferred sites for adsorption of CO_2 by protein in the gas-solid phase system. Cundari et al. (2009) analysed the binding of CO_2 to proteins utilizing a combination of bioinformatics, molecular modelling, and first-principles quantum mechanics, and concluded that the hydrogen bonds between the functional groups of the amino acids and the oxygen sites on the carbon dioxide were involved in the CO_2 adsorption process.

The relationship between moisture-to-protein ratio and CO_2 solubility was non-linear (Fig. 4), and can be divided into three distinct regions: (1) a rapid decrease in CO_2 solubility on increasing the moisture-to-protein ratio from ~0.03 to ~0.5; (2) a relatively slower decrease in CO_2 solubility with increasing moisture-to-protein ratio from ~0.5 to ~1.7; and (3) a small but significant increase in CO_2 solubility on increasing moisture-to-protein ratio from ~1.7 to ~2.5. Around a 4-fold decrease in CO_2 solubility was observed when the average moisture-to-protein ratio of casein matrices increased from ~0.03 to 0.5. Mitsuda et al. (1975) also reported similar solubility behaviour of CO_2 in dried casein and gelatine powder as a function of moisture content. The solubility of CO_2 in casein or gelatine powder decreased by >90% when their moisture content increased from ~5 or 10% (w/w) to 20 or 40% (w/w). The authors also observed a rapid decrease in CO_2 solubility of casein or gelatine powder prior to a gradual decrease in their CO_2 solubility on increasing moisture levels. Pre-adsorbed water may interact with the reactive sites of casein matrices making those reactive sites unavailable for interaction with CO_2, thus decreasing the CO_2 adsorption/solubilisation capacity of hydrated casein matrices. A similar moisture-dependent CO_2 adsorption behaviour has also been observed in other non-food materials. For example, Ozdemir and Schroeder (2009) observed a lower CO_2 adsorption capacity of wet coals compared to that of dried coals. Those authors speculated that the adsorbed water occupies the pore spaces or the active sites for the adsorption of CO_2.

Water in casein matrices is either present in a bulk (freezable at – 40 °C) or bound form (non-freezable at – 40 °C) (P. Lamichhane, Kelly, & Sheehan, 2018b; McMahon et al., 1999), and the latter is typically considered to be so-called primary hydration water and primarily related to the solvation of polar and charged residues (Huppertz et al., 2017). In casein matrices of moisture-to-protein ratio up to 0.5, almost all moisture (> 98% of total moisture) was found to be in non-freezable form (Fig. 4). This result further supports the hypothesis that the pre-adsorbed water may interact with the reactive sites (e.g., polar and charged residues) of casein matrices, making those reactive sites unavailable for CO_2 interaction.

The solubility of CO_2 in casein matrices first decreased ($P < 0.05$) by ~23% with increasing moisture-to-protein ratio from ~0.5 to ~1.7 and then increased ($P < 0.05$) by ~21% with increasing moisture-to-protein ratio from ~1.7 to ~2.5 (Fig. 4, inset). Such complex relationships observed between CO_2 solubility and moisture-to-protein ratio may be attributed to interactive effects of moisture and protein content on CO_2 solubility. This suggests that both water and protein components of casein matrices have an important role on CO_2 solubility.

In eye-type cheeses, the moisture-to-protein ratio is between 1.2 and 2.0, e.g., ~1.8 in Maasdam (P. Lamichhane, Kelly, et al., 2018a; P. Lamichhane, Pietrzyk, et al., 2018), ~1.7 in Edam and ~1.25 in Emmental (Deegan et al., 2013). Therefore, the solubility of CO_2 studied in casein matrices with a protein-to-moisture ratio between 1.0 and 2.0 are particularly important for hard and semi-hard eye-type cheeses. Moisture-to-protein ratios in cheese may also vary on a batch-to-batch basis, due to seasonal variations in the composition of milk and thus the resultant cheeses. Therefore, these results could form the basis for development of a robust model for prediction of CO_2 solubility in a wide variety of cheeses of different compositions, as models reported in previous studies were limited to the cheese type under study (Acerbi et al., 2016; Jakobsen et al., 2009).

Although the solubility behaviour of CO_2 studied in casein matrices with a protein-to-moisture ratio below 1.0 is not relevant to natural cheese matrices, such knowledge may be useful when designing modified atmosphere packaging for cheese powders, where the protein to moisture ratio in spray-dried cheese powders was reported to vary between 0.05 and 0.12 (Felix da Silva, Larsen, Hougaard, & Ipsen, 2017).

Various approaches used in this study to achieve different moisture-to-protein ratios could potentially lead to differences in microstructure of the casein matrices. However, microstructure seems to have a low influence on the solubility of CO_2. For example, Jakobsen et al. (2006) did not observe a significant difference in the amount of CO_2 adsorbed between meat samples (i.e., whole versus minced meat) having similar compositions but different microstructures. Further research is recommended to elucidate the influence of microstructure on the solubility of CO_2.

4. Conclusions

This study investigated the solubility behaviour of CO_2 in casein matrices, representing varying conditions of the protein-water phase of semi-hard cheese matrices. Both compositional (i.e, moisture-to-protein ratio and salt-in-moisture content) and ripening-related (i.e., pH and temperature) parameters had a significant influence on CO_2 solubility of casein matrices. Variation in the cheese composition from batch to batch due to differences in milk composition or manufacture derived variables, such as time of the day of manufacture, plant temperature, temperature of brine and brining times, and rennet-to-casein ratio, may result in certain batches being more at risk for development of slits and cracks.

Overall, the result obtained from this study should form the basis for development of a robust model for CO_2 solubility in a wide variety of cheese types or where the composition of cheese may vary within a commercial cheese production plant. Such knowledge may help to improve the quality and consistency of eye-type cheese by minimizing or avoiding development of slits and cracks.

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References

Acerbi, F., Guillard, V., Guillaume, C., & Gontard, N. (2016). Impact of selected composition and ripening conditions on CO_2 solubility in semi-hard cheese. *Food Chemistry, 192*, 805-812. https://doi.org/10.1016/j.foodchem.2015.07.049.

Adhikari, B. M., Truong, T., Bansal, N., & Bhandari, B. (2018). Use of gases in dairy manufacturing: A review. *Critical Reviews in Food Science and Nutrition, 58*, 2557-2569. https://doi.org/10.1080/10408398.2017.1333488.

Bachu, S., & Bennion, D. B. (2009). Interfacial tension between CO_2, freshwater, and brine in the range of pressure from (2 to 27) MPa, temperature from (20 to 125) °C, and water salinity from (0 to 334 000) $mg\cdot L^{-1}$. *Journal of Chemical and Engineering Data, 54*, 765-775. https://doi.org/10.1021/je800529x.

Bisig, W., Guggisberg, D., Jakob, E., Turgay, M., Irmler, S., Wechsler, D., & Fröhlich-Wyder, M.-T. (2019). The effect of NaCl and metabolic profile of propionibacteria on eye formation in experimental Swiss-type cheese. *International Dairy Journal, 89*, 86-95. https://doi.org/10.1016/j.idairyj.2018.09.001.

Burdikova, Z., Svindrych, Z., Pala, J., Hickey, C. D., Wilkinson, M. G., Panek, J., Auty, M. A. E., Periasamy, A., & Sheehan, J. J. (2015). Measurement of pH micro-heterogeneity in natural cheese matrices by fluorescence lifetime imaging. *Frontiers in microbiology, 6*, 183. https://doi.org/10.3389/fmicb.2015.00183.

Carvalho, P. J., Pereira, L. M. C., Gonçalves, N. P. F., Queimada, A. J., & Coutinho, J. A. P. (2015). Carbon dioxide solubility in aqueous solutions of NaCl: Measurements and modeling with electrolyte equations of state. *Fluid Phase Equilibria, 388*, 100-106. https://doi.org/10.1016/j.fluid.2014.12.043.

Cofie-Agblor, R., Muir, W. E., Sinicio, R., Cenkowski, S., & Jayas, D. S. (1995). Characteristics of carbon dioxide sorption by stored wheat. *Journal of Stored Products Research, 31*, 317-324. https://doi.org/10.1016/0022-474X(95)00022-Y.

Cundari, T. R., Wilson, A. K., Drummond, M. L., Gonzalez, H. E., Jorgensen, K. R., Payne, S., Braunfeld, J., De Jesus, M., & Johnson, V. M. (2009). CO_2-formatics: How do proteins bind carbon dioxide? *Journal of Chemical Information and Modeling, 49*, 2111-2115. https://doi.org/10.1021/ci9002377.

Daly, D. F. M., McSweeney, P. L. H., & Sheehan, J. J. (2010a). Investigation of underlying factors in split and secondary fermentation defects in rindless block Swiss-type cheeses. *Australian Journal of Dairy Technology, 65*, 214-216.

Daly, D. F. M., McSweeney, P. L. H., & Sheehan, J. J. (2010b). Split defect and secondary fermentation in Swiss-type cheeses – A review. *Dairy Science & Technology, 90*, 3-26. https://doi.org/10.1051/dst/2009036.

Deegan, K. C., Heikintalo, N., Ritvanen, T., Putkonen, T., Rekonen, J., McSweeney, P. L. H., Alatossava, T., & Tuorila, H. (2013). Effects of low-pressure homogenisation on the sensory and chemical properties of Emmental cheese. *Innovative Food Science & Emerging Technologies, 19*, 104-114. https://doi.org/10.1016/j.ifset.2013.04.008.

Dixon, N. M., & Kell, D. B. (1989). The control and measurement of 'CO2' during fermentations. *Journal of Microbiological Methods, 10*, 155-176. https://doi.org/10.1016/0167-7012(89)90048-1.

Felix da Silva, D., Larsen, F. H., Hougaard, A. B., & Ipsen, R. (2017). The influence of raw material, added emulsifying salt and spray drying on cheese powder structure and hydration properties. *International Dairy Journal, 74*, 27-38. https://doi.org/10.1016/j.idairyj.2017.01.005.

Gill, C. O. (1988). The solubility of carbon dioxide in meat. *Meat Science, 22*, 65-71. https://doi.org/10.1016/0309-1740(88)90027-7.

Govindasamy-Lucey, S., Jaeggi, J., Martinelli, C., Johnson, M., & Lucey, J. (2011). Standardization of milk using cold ultrafiltration retentates for the manufacture of Swiss cheese: Effect of altering coagulation conditions on yield and cheese quality. *Journal of Dairy Science, 94*, 2719-2730. https://doi.org/10.3168/jds.2010-3842.

Guinee, T. P. (2004). Salting and the role of salt in cheese. *International Journal of Dairy Technology, 57*, 99-109. https://doi.org/10.1111/j.1471-0307.2004.00145.x.

Holzammer, C., Finckenstein, A., Will, S., & Braeuer, A. S. (2016). How sodium chloride salt inhibits the formation of CO_2 gas hydrates. *The Journal of Physical Chemistry B, 120*, 2452-2459. https://doi.org/10.1021/acs.jpcb.5b12487.

Huppertz, T., Gazi, I., Luyten, H., Nieuwenhuijse, H., Alting, A., & Schokker, E. (2017). Hydration of casein micelles and caseinates: Implications for casein micelle structure. *International Dairy Journal, 74*, 1-11. https://doi.org/10.1016/j.idairyj.2017.03.006.

IDF. (1996). Milk. Determination of fat content (Röse Gottlieb gravimetric method). International Dairy Federation, Brussels, Belgium.

Jakobsen, M., & Bertelsen, G. (2002). The use of CO_2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: A review. *Journal of Muscle Foods, 13*, 143-168. https://doi.org/10.1111/j.1745-4573.2002.tb00326.x.

Jakobsen, M., & Bertelsen, G. (2006). Solubility of carbon dioxide in fat and muscle tissue. *Journal of Muscle Foods, 17*, 9-19. https://doi.org/10.1111/j.1745-4573.2006.00029.x.

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*, 287-294. https://doi.org/10.1007/s00217-009-1059-3.

Lamichhane, P. (2019). *Influence of milk pre-treatment, and changes in cheese structure and carbon dioxide solubility on the development of split defects in cheese.* PhD thesis, University College Cork.

Lamichhane, P., Auty, M. A. E., Kelly, A. L., & Sheehan, J. J. (2020). Dynamic in situ imaging of semi-hard cheese microstructure under large-strain tensile deformation: Understanding structure-fracture relationships. *International Dairy Journal, 103*, 104626. https://doi.org/10.1016/j.idairyj.2019.104626.

Lamichhane, P., Kelly, A. L., & Sheehan, J. J. (2018a). Effect of milk centrifugation and incorporation of high-heat-treated centrifugate on the composition, texture, and ripening characteristics of Maasdam cheese. *Journal of Dairy Science, 101*, 5724-5737. https://doi.org/10.3168/jds.2017-14178.

Lamichhane, P., Kelly, A. L., & Sheehan, J. J. (2018b). Symposium review: Structure-function relationships in cheese. *Journal of Dairy Science, 101*, 2692-2709. https://doi.org/10.3168/jds.2017-13386.

Lamichhane, P., Pietrzyk, A., Feehily, C., Cotter, P. D., Mannion, D. T., Kilcawley, K. N., Kelly, A. L., & Sheehan, J. J. (2018). Effect of milk centrifugation and incorporation of high heat-treated centrifugate on the microbial composition and levels of volatile organic compounds of Maasdam cheese. *Journal of Dairy Science, 101*, 5738-5750. https://doi.org/10.3168/jds.2017-14180.

Lamichhane, P., Sharma, P., Kennedy, D., Kelly, A. L., & Sheehan, J. J. (2019). Microstructure and fracture properties of semi-hard cheese: Differentiating the effects of primary proteolysis and calcium solubilization. *Food Research International, 125*, 108525. https://doi.org/10.1016/j.foodres.2019.108525.

McMahon, D. J., Fife, R. L., & Oberg, C. J. (1999). Water partitioning in Mozzarella cheese and its relationship to cheese meltability. *Journal of Dairy Science, 82*, 1361-1369. https://doi.org/10.3168/jds.S0022-0302(99)75361-0.

Mitsuda, H., Kawa, F., Yamamoto, A., & Nakajima, K. (1975). Carbon dioxide-protein interaction in a gas-solid phase. *Journal of Nutritional Science and Vitaminology*, *21*, 151-162. https://doi.org/10.3177/jnsv.21.151.

Mitsuda, H., Kawai, F., Yamamoto, A., Suzuki, F., Nakajima, K., & Yasumoto, K. (1977). Identification and properties of reactive sites in protein capable of binding carbon dioxide in a gas-solid phase system. *Journal of Nutritional Science and Vitaminology*, *23*, 145-152. https://doi.org/10.3177/jnsv.23.145.

Ozdemir, E., & Schroeder, K. (2009). Effect of moisture on adsorption isotherms and adsorption capacities of CO_2 on coals. *Energy & Fuels*, *23*, 2821-2831. https://doi.org/10.1021/ef801126a.

Salaün, F., Mietton, B., & Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal*, *15*, 95-109. https://doi.org/10.1016/j.idairyj.2004.06.007.

Sheehan, J. J., Fenelon, M. A., Wilkinson, M. G., & McSweeney, P. L. H. (2007). Effect of cook temperature on starter and non-starter lactic acid bacteria viability, cheese composition and ripening indices of a semi-hard cheese manufactured using thermophilic cultures. *International Dairy Journal, 17*, 704-716. https://doi.org/10.1016/j.idairyj.2006.08.011.

Singh, P., Wani, A. A., Karim, A. A., & Langowski, H.-C. (2012). The use of carbon dioxide in the processing and packaging of milk and dairy products: A review. *International Journal of Dairy Technology, 65*, 161-177. https://doi.org/10.1111/j.1471-0307.2011.00744.x.

Sivertsvik, M., & Jensen, J. S. (2005). Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 3: Cooked meat products. *Journal of Food Engineering, 70,* 499-505. https://doi.org/10.1016/j.jfoodeng.2004.10.005.

Sivertsvik, M., Rosnes, J. T., & Jeksrud, W. K. (2004). Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 2: Raw fish fillets. *Journal of Food Engineering, 63*, 451-458. https://doi.org/10.1016/j.jfoodeng.2003.09.004.

Truong, T., Palmer, M., Bansal, N., & Bhandari, B. (2017). Investigation of solubility of carbon dioxide in anhydrous milk fat by lab-scale manometric method. *Food Chemistry, 237*, 667-676. https://doi.org/10.1016/j.foodchem.2017.05.141.

Walstra, P., Wouters, J. T., & Geurts, T. J. (2005). *Dairy Science and Technology*. Boca Raton, FL, USA: CRC press.

Figure legends

Fig. 1. (a) Carbon dioxide concentration in casein matrices as a function of carbon dioxide partial pressure in the headspace of modified atmosphere packages and temperature; (○) 5 °C, (□) 12 °C, and (◊) 25 °C; error bars represent standard deviations of means (n = 3). (b) Carbon dioxide concentration in the casein matrices as a function of temperature; (▲), mean of data from three replicate experiments; (●), individual data point; *P* represents P-value; error bars represent standard deviations of means (n = 3). Average composition (± standard deviation) of samples: moisture content = 61.43±1.32 (%, w/w), protein content = 31.20±0.05 (%, w/w), fat content: 0.39±0.10 (%, w/w), salt content = 1.90±0.05 (%, w/w), salt-in-moisture content = 3.09±0.05 (%, w/w), and pH = 5.84±0.02.

Fig. 2. Effect of salt-in-moisture content on the solubility of carbon dioxide in casein matrices; (▲), mean of data from three replicate experiments; (●), individual data point; *P* represent P-value. Error bars represent standard deviations of means (n = 3). Average composition (± standard deviation) of samples: moisture content = 61.38±1.30 (%, w/w), protein content = 31.11±1.78 (%, w/w), fat content = 0.39±0.08 (%, w/w), and pH = 5.37±0.03.

Fig. 3. Effect of pH on carbon dioxide solubility in the casein matrices; (▲), mean of data from three replicate experiments; (●), individual data point; *P* represent P-value. Error bars represent standard deviation of mean (n = 3). Average composition (± standard deviation) of samples: moisture content = 61.05±0.9 (%, w/w), protein content = 31.81±0.96 (%, w/w), fat content = 0.39±0.08 (%, w/w), and salt content = 1.92±0.03 (%, w/w), salt-in-moisture content = 3.14±0.08 (%, w/w).

Fig. 4. Relationships between moisture-to-protein ratio and (▲) CO_2 solubility or (■) non-freezable moisture (% of the total moisture) in renneted-casein matrices; (●) individual data point. Inset: magnification of CO_2 solubility data for casein matrices of moisture-to-protein

ratio between 0.5 and 2.5; means with different letters differ ($P < 0.05$). Error bars represent standard deviations of means (n = 3).

Prabin Lamichhane: Conceptualization, Methodology, Writing - Original Draft, Visualization, Investigation, Formal analysis, Validation.

Prateek Sharma: Methodology.

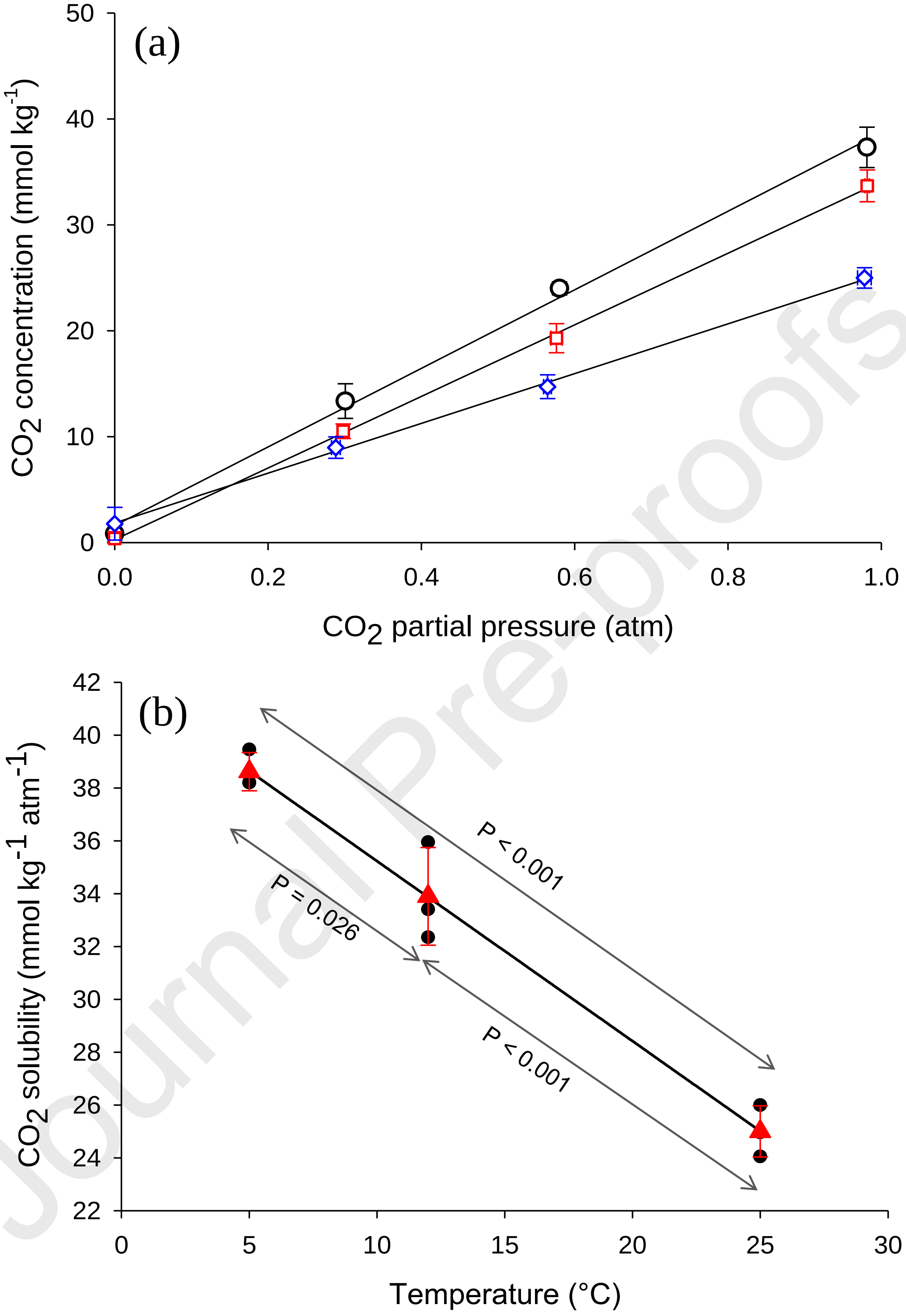
Alan L. Kelly: Writing - Review & Editing, Supervision.

Jens Risbo: Methodology, Conceptualization, Resources, Supervision.

Fergal P. Rattray: Conceptualization, Resources, Supervision.

Jeremiah J. Sheehan: Conceptualization, Supervision, Writing - Review & Editing, Project administration, Funding acquisition.

Declaration of Interest Statement: None



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583 Fig. 1

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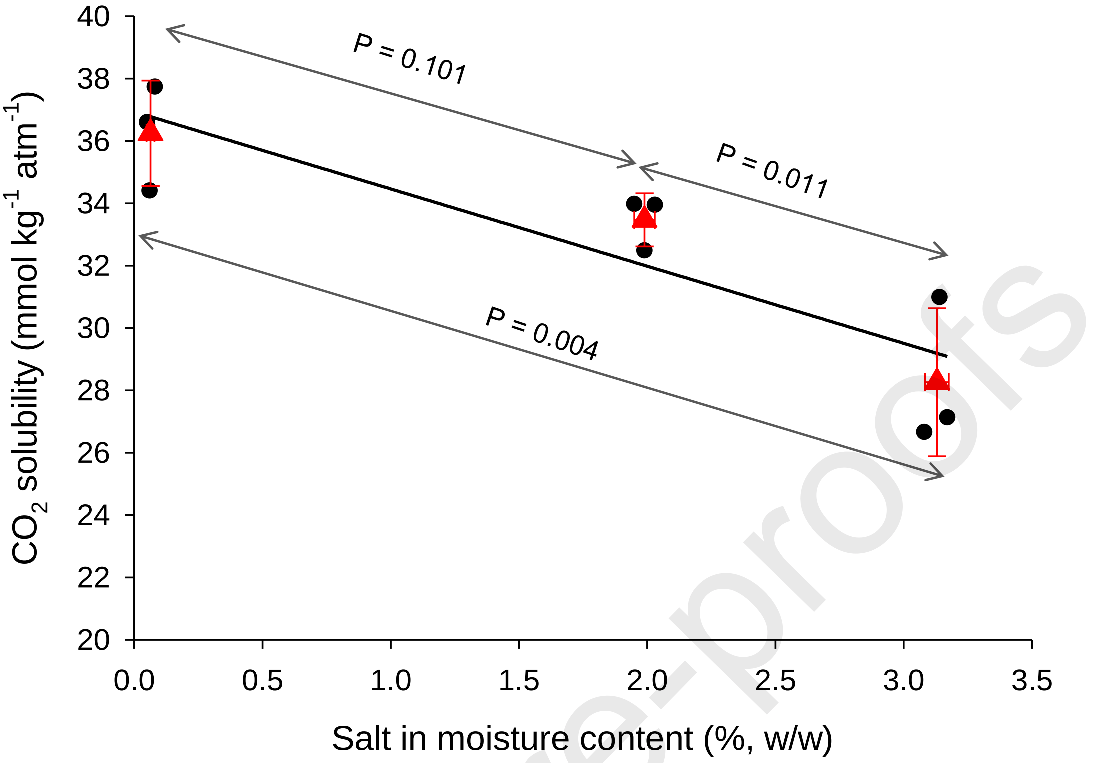
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2.5
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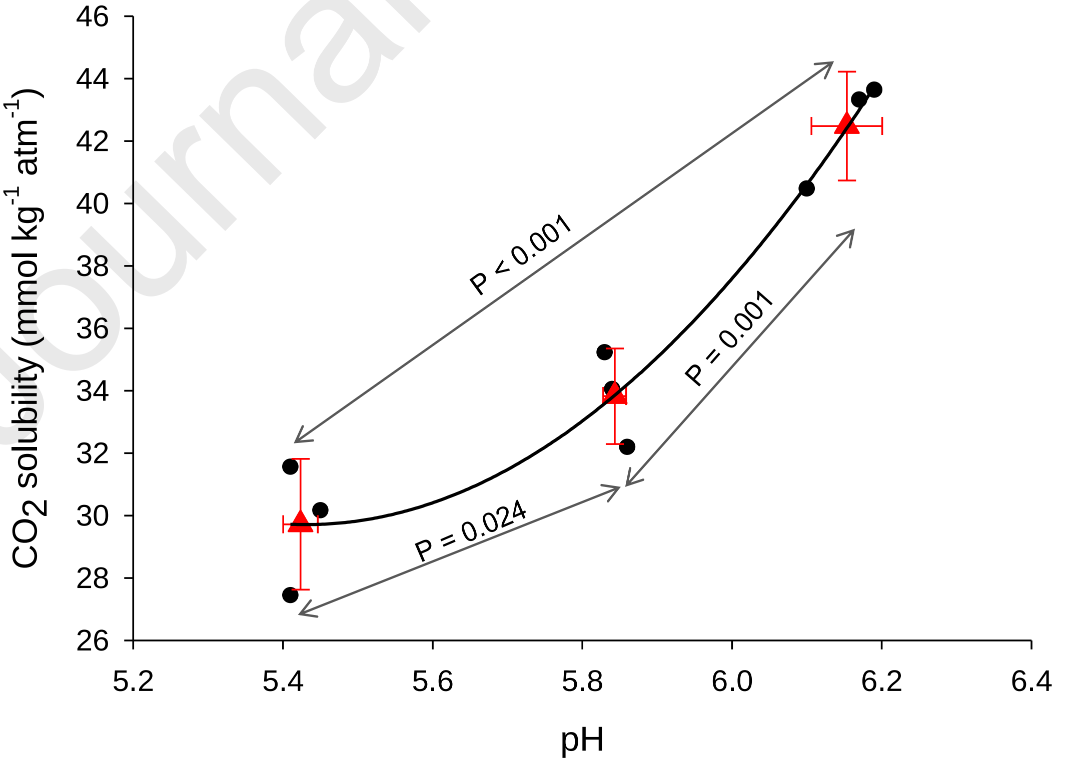
CO2 solubility (mmol kg-1 atm-1)
Salt in moisture content (%, w/w)
P = 0.101
P = 0.011
P = 0.004

Fig. 2.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5.2
5.4
5.6
5.8
6.0
6.2
6.4
CO2 solubility (mmol kg-1 atm-1)
pH
P < 0.001
P = 0.001
P = 0.024

Fig. 3.

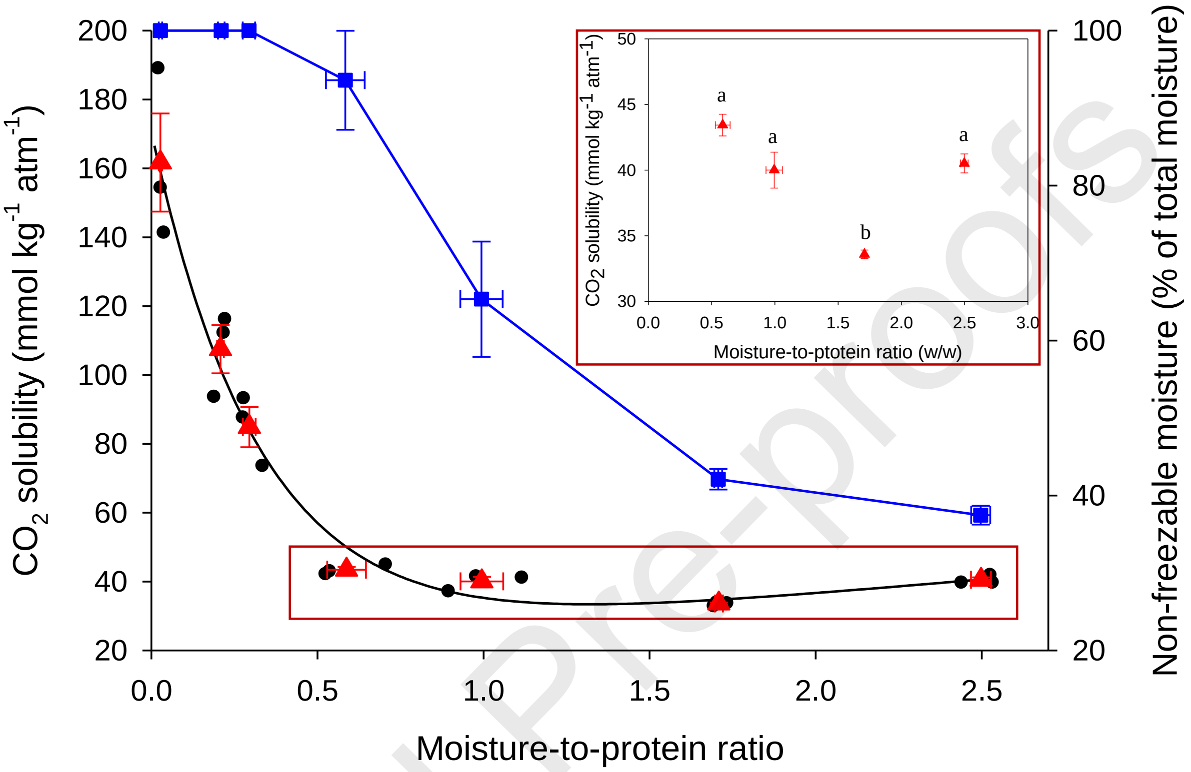

Fig. 4.

Highlights

- Model casein matrices with varying moisture-to-protein ratio, pH, and salt content were prepared
- The CO$_2$ solubility of samples decreased with increasing temperature and salt-in-moisture content
- A non-linear relationship was observed between CO$_2$ solubility and the moisture-to-protein ratio

- Controlling variability in cheese compositional and ripening temperatures will reduce the incidence of slit defects