

Journal Pre-proofs

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

Prabin Lamichhane, Prateek Sharma, Alan L. Kelly, Jens Risbo, Fergal P. Rattray, Jeremiah J. Sheehan

PII: S0308-8146(20)31487-4

DOI: <https://doi.org/10.1016/j.foodchem.2020.127625>

Reference: FOCH 127625

To appear in: *Food Chemistry*

Received Date: 28 February 2020

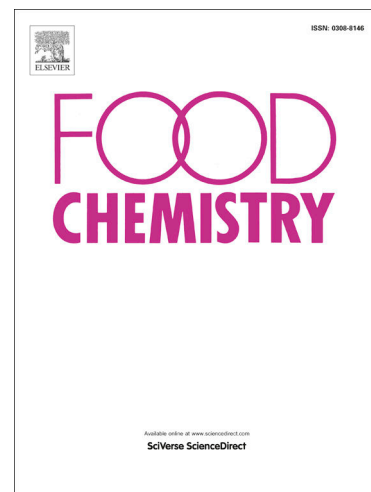
Revised Date: 1 July 2020

Accepted Date: 18 July 2020

Please cite this article as: Lamichhane, P., Sharma, P., Kelly, A.L., Risbo, J., Rattray, F.P., Sheehan, J.J., Solubility of carbon dioxide in renneted casein matrices: Effect of pH, salt, temperature, partial pressure, and moisture to protein ratio, *Food Chemistry* (2020), doi: <https://doi.org/10.1016/j.foodchem.2020.127625>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Elsevier Ltd. All rights reserved.



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

3 Prabin Lamichhane^{a,b}, Prateek Sharma^{a,1}, Alan L. Kelly^b, Jens Risbo^c, Fergal P. Rattray^c, Jeremiah J.
4 Sheehan^{a,*}

5 ^aTeagasc Food Research Centre, Moorepark, Fermoy, Cork, P61 C996, Ireland

6 ^bSchool of Food and Nutritional Sciences, University College Cork, T12 YN60, Ireland

7 ^cDepartment of Food Science, University of Copenhagen, Rolighedsvej 26, DK-1958, Denmark

8

9

10 *Corresponding author at: Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland P61
11 C996

12 Email: diarmuid.sheehan@teagasc.ie

13

14 ¹Present address: Department of Nutrition, Dietetics, and Food Science, Utah State
15 University, Logan, UT, USA

16 **Abstract**

17 The solubility of carbon dioxide (CO₂) in the moisture and protein components of
18 cheese matrices and the influence of changing pH, salt and temperature levels remains
19 unclear. In this study, model casein matrices were prepared, by renneting of micellar casein
20 concentrate (MCC), with modulation of salt and pH levels by adding salt and glucono delta-
21 lactone, respectively to the MCC solutions prior to renneting. Different moisture-to-protein
22 levels were achieved by freeze-drying, incubation of samples at different relative humidities,
23 or by applying varying pressures during gel manufacture. The CO₂ solubility of samples
24 decreased linearly with both increasing temperature and salt-in-moisture content, whereas
25 solubility of CO₂ increased with increasing pH. A non-linear relationship was observed
26 between CO₂ solubility and the moisture-to-protein ratio of experimental samples. Overall,
27 such knowledge may be applied to improve the quality and consistency of eye-type cheese,
28 and in particular to avoid development of undesirable slits and cracks.

29
30
31 **Keywords:** Carbon dioxide solubility; Casein matrices; Slit and crack defects; Eye-
32 development

33 1. Introduction

34 In some eye-type cheeses, such as Emmental and Maasdam, propionic acid bacteria
35 produce a high level of carbon dioxide, especially during warm-room ripening. The rate and
36 extent of gas production and its behaviour in the cheese matrix (e.g., solubility and
37 diffusivity) are considered important factors for the development of eyes, but are also
38 implicated in the undesired development of slits or cracks within those cheese types (Daly,
39 McSweeney, & Sheehan, 2010b; P. Lamichhane, 2019). It is believed that CO₂ produced
40 within the cheese matrix first solubilizes/dissolves within the components of the cheese
41 matrix. Once the cheese body becomes saturated with gas, it then diffuses to the nuclei for
42 eye-development or diffuses outward through the cheese rind. It has been reported that ~50%
43 of the total CO₂ gas produced is dissolved in the cheese body (Walstra, Wouters, & Geurts,
44 2005).

45 Studies have suggested that the CO₂ solubility within the cheese matrix largely
46 depends on factors such as cheese composition, temperature, and partial pressure. Carbon
47 dioxide solubilizes in both the aqueous and fat phases of cheese; however, the CO₂ solubility
48 capacity of each phase is temperature-dependent (Jakobsen, Jensen, & Risbo, 2009). CO₂
49 solubility in the aqueous phase of cheese has been reported to decrease with increasing
50 temperature whereas the CO₂ solubility in the fat phase has been reported to increase with
51 increasing temperature (Jakobsen et al., 2009). Acerbi, Guillard, Guillaume, and Gontard
52 (2016) studied the effect of temperature, partial pressure, salt and moisture content on the
53 solubility behaviour of CO₂ in semi-hard cheese. Those authors observed a decrease in CO₂
54 solubility with increasing temperature and salt level. However, a complex relationship was
55 observed with moisture level, which has been attributed to concomitant changes in protein
56 content with changing moisture levels. Those authors recommended conducting further
57 research to clarify the influence of nitrogen content on CO₂ solubility in cheese.

58 Although several studies have investigated CO₂ solubility behaviour in food matrices
59 (Acerbi et al., 2016; Adhikari, Truong, Bansal, & Bhandari, 2018) or in pure fat (Jakobsen et
60 al., 2009; Truong, Palmer, Bansal, & Bhandari, 2017), the solubility behaviour of CO₂ in
61 dairy protein matrices is not yet fully understood. In fact, studies have neglected the effect of
62 protein content on CO₂ solubility (Acerbi et al., 2016; Jakobsen et al., 2009). However, it is
63 difficult to investigate the effect of each individual component on solubility behaviour in a
64 multi-component food system, as changing of one compositional parameter results in
65 consequential changes to other compositional parameters. Therefore, studies using model
66 systems may be better suited to understand the effect of each component individually on
67 solubility behaviour of CO₂.

68 The primary aim of this study was to investigate the effect of moisture-to-protein ratio
69 on the solubility of CO₂ in renneted-casein gel matrices rather than simply in a protein-only
70 matrix, as it is not possible to vary protein content without changing the moisture level. In the
71 majority of food matrices, protein is mostly present in a hydrated state, and its level of
72 hydration is dependent on product type; for example, there are low levels of protein hydration
73 in dairy powders compared to cheese. An additional aim of this study was to elucidate the
74 effect of varying levels of salt, pH, temperature and partial pressure on the solubility of CO₂
75 in model renneted-casein gel matrices.

76 **2. Materials and methods**

77 *2.1. Preparation of renneted casein matrix*

78 Liquid micellar casein concentrate (MCC; protein content = 14.55%, w/w; total
79 solids: 18.34%, w/w) was produced as reported by Xia et al. (accepted for publication,
80 International Dairy Journal, article number: 104796) and stored at -18 °C. Prior to use in
81 experiments, the MCC was thawed in a water bath at 50 °C and an aliquot (400 g) was placed

82 in a 500 mL beaker with 0.03 % (w/w) sodium azide (BDH Chemicals, Poole, England) as a
83 preservative. The desired salt concentration and pH levels of the final gels, were achieved by
84 mixing varying levels of NaCl (0, 1.5, or 2.5%, w/w) and glucono- δ -lactone (GDL; 0.5, 1.2,
85 or 2%, w/w; Sigma-Aldrich) into the MCC with a magnetic stirrer. Three minutes after salt
86 and GDL addition, fermentation-produced bovine chymosin (FPBC; CHY-MAX Plus, ~200
87 international milk clotting units (IMCU)/mL; Chr. Hansen Ltd., Cork, Ireland) was added at a
88 level of 0.82 mL kg⁻¹ MCC. Rennet addition was based on MCC protein content. All renneted
89 milk concentrates were incubated at 32 °C for 30 min to induce gel formation and stored
90 overnight at 4 °C for completion of GDL hydrolysis.

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

96 *2.1.1 Preparation of a model system to investigate the effect of partial pressure and* 97 *temperature*

98 To investigate the effect of partial pressure and temperature on CO₂ solubility, three
99 identical casein matrices were prepared of moisture and salt content ~60% (w/w) and ~2%
100 (w/w), respectively, and with a pH of ~5.8.

101 *2.1.2 Preparation of a model system to investigate the effect of salt*

102 To investigate the effect of salt on the CO₂ solubility, three casein matrices were
103 prepared in triplicate, by adding three different salt levels, i.e., 0, 1.5, and 2.5%, w/w, to the
104 MCC. Other parameters, including levels of protein and moisture, and pH, were all kept
105 constant.

106 2.1.3 Preparation of a model system to investigate the effect of pH

107 To investigate the effect of pH on the CO₂ solubility, three casein matrices, of three
108 different pHs, were prepared in triplicate, by adding varying levels of GDL (0.5, 1.2, or 2%,
109 w/w) to the MCC. Other parameters, including levels of salt, protein and moisture, were all
110 kept constant. A series of preliminary experiments were initially conducted to determine the
111 levels of GDL necessary to achieve the desired pH value, which ranged between 5.4 and 6.2.

112 2.1.4 Preparation of a model system to investigate the effect of moisture-to-protein ratio

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

126 2.2. Composition analysis

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

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

131 *2.3. Determination of freezable and non-freezable moisture*

132 Levels of freezable and bound moisture were determined using a differential scanning
133 calorimeter (DSC; Q200, TA Instruments, New Castle, DE, USA) as described by McMahon,
134 Fife, and Oberg (1999). Freezable moisture is defined as water freezable at $-40\text{ }^{\circ}\text{C}$, whereas
135 non-freezable moisture was defined as the water that did not freeze at $-40\text{ }^{\circ}\text{C}$ (McMahon et
136 al., 1999).

137 *2.4. Modified atmosphere packaging*

138 In triplicate, experimental samples of 1.5 to 2 g, were each individually packaged into
139 pouches (length = 25 cm, width = 18.5 cm; Amcor Flexibles, Denmark) of high gas
140 impermeability using a modified atmosphere packaging machine equipped with a two-gas
141 mixture (A300; Multivac, Germany). To investigate the effect of partial pressure and
142 temperature on CO_2 solubility, samples were packed with a $\text{CO}_2:\text{N}_2$ gas mixture of 0:100;
143 30:70; 60:40 and 100:0 and stored at $5\text{ }^{\circ}\text{C}$, $12\text{ }^{\circ}\text{C}$ and $25\text{ }^{\circ}\text{C}$ for 2 d. To investigate the effect
144 of salt, pH and casein hydration, samples were packed under 100% CO_2 and stored at $5\text{ }^{\circ}\text{C}$
145 for 2 d.

146 *2.5. Determination of the concentration of CO_2 in the headspace of modified atmosphere* 147 *packages*

148 The concentration of CO_2 in the headspace of modified atmosphere packages was
149 determined using a headspace gas analyser (CheckMate 9900, PBI-Dansensor A/S, Ringsted,
150 Denmark). To prevent gas leakage from the packaging material during measurement, a

151 septum (diameter = 15 mm, MACON Europe A/S, Denmark) was attached to the top of the
152 packaging materials which was pierced by the needle of the headspace gas analyser.

153 2.6. Determination of the concentration of CO₂ in renneted-casein matrices

154 The concentration of carbon dioxide in the renneted-casein matrix was determined
155 using a titration method as described in previous studies (Gill, 1988; Jakobsen et al., 2009;
156 Truong et al., 2017). Briefly, a pair of side-armed conical flasks (100 mL; Pyrex), one
157 containing 10 mL of 0.5 M H₂SO₄ and another containing 3 mL of 0.1 N Ba(OH)₂,
158 connected by a reinforced PVC tube, were used for extraction and subsequent scavenging of
159 CO₂ from experimental samples. Experimental samples (~1.5 g) held under modified
160 atmosphere packaging were transferred immediately to the flask containing 0.5 M H₂SO₄,
161 which was sealed using a neoprene stopper. Therein, the CO₂ released from the experimental
162 sample reacted with Ba(OH)₂ (present in the other flask) and produced a BaCO₃ precipitate.
163 After at least 24 h, the residual Ba(OH)₂ was titrated against a standard HCl solution (0.1 M)
164 using phenolphthalein as an indicator.

165 2.7. Statistical analysis

166 Statistical analyses of the data were performed using SigmaPlot version 14 (Systat
167 Software, Inc., San Jose, California, USA). The effect of treatment on CO₂ solubility of
168 casein matrices was determined performing one way ANOVA followed by *post-hoc* Student-
169 Newman-Keuls tests. Before ANOVA evaluation, data were checked for homoscedasticity
170 and normality by performing Brown-Forsythe and Shapiro–Wilk tests, respectively. The level
171 of significance was set at $P \leq 0.05$. Regression analyses of the data were performed using
172 SigmaPlot version 14 (Systat Software, Inc., San Jose, California, USA).

173 3. Results and discussions

174 3.1. Effects of partial pressure and temperature on solubility of CO₂

175 A linear relationship was observed between the concentrations of CO₂ in the
176 experimental samples and the CO₂ partial pressure of the headspace of corresponding
177 samples at all three temperatures investigated (Fig. 1a). These results are in agreement with
178 previous studies on cheese (Acerbi et al., 2016; Jakobsen et al., 2009) and on anhydrous milk
179 fat (Truong et al., 2017). The linear regression equations obtained had very high coefficients
180 of determination ($R^2 = 0.98-0.99$), thus validating Henry's law for the casein matrix studied.
181 However, a small deviation from the origin was observed at zero partial pressure of CO₂.
182 This deviation in CO₂ solubility of samples ranged between 0 and 2.68 mmol kg⁻¹. Similar
183 deviations from the origin have previously been observed in cheese (Acerbi et al., 2016;
184 Jakobsen et al., 2009) and in anhydrous milk fat (Truong et al., 2017) and were attributed to
185 the inherent presence of carbamate or carbonate species within the sample (Acerbi et al.,
186 2016; Jakobsen et al., 2009). We believe that such a small offset plays a negligible role in the
187 overall determination of the relationship between the physicochemical parameters of the
188 matrix and CO₂ solubility.

189 The solubility of CO₂ in a casein matrix decreased ($P < 0.05$) linearly ($R^2 = 0.96$) as
190 the temperature increased, with ~35% lower CO₂ solubility observed at 25 °C than at 5 °C
191 (Fig. 1b). It is proposed that the random molecular motion of the CO₂ gas molecules
192 increases with increasing temperature (Cofie-Agblor, Muir, Sinicio, Cenkowski, & Jayas,
193 1995), thereby reducing the forces of attraction between CO₂ gas and the casein matrix, with
194 subsequent release of CO₂ from the casein matrix.

195 Slits and cracks are usually observed during cold room storage and, therefore, we
196 propose that the changes in CO₂ solubility with changing ripening temperature may

197 contribute to the occurrence of such defects. Cheeses such as Maasdam and Emmental are
198 pre-ripened for 1 to 2 weeks at 8-10 °C before warm room ripening (~23 °C) for 4 to 6 weeks
199 for the development of eyes, and are finally stored at 2-4 °C. Propionic acid bacteria produce
200 a high level of CO₂, especially during warm-room ripening, and a proportion (~50%) of CO₂
201 produced dissolves in the cheese body, while the remainder diffuses to nuclei for eye-
202 formation (~20%) and diffuses outward (~30%) through the cheese rind. During ripening, the
203 cheese texture become more brittle (i.e., fracturing of cheese matrix at a relatively small
204 deformation), most probably due to solubilisation of colloidal calcium, hydrolysis of caseins,
205 or both (P. Lamichhane, Sharma, Kennedy, Kelly, & Sheehan, 2019). Moreover, the cheese
206 texture also becomes brittle during cold storage due to solidification of fat. This suggests that
207 sudden and significant increase in cheese storage temperature due to a range of
208 circumstances, such as increased external ambient temperature or the arrival of new batches
209 of cheese (from warm rooms) into the cold rooms decrease the gas solubility and this
210 increases the CO₂ partial pressure within the cheese matrix. Such unintended increases in
211 partial pressure at later stages of ripening (i.e., during cold storage temperature) may
212 overcome the adhesive or cohesive strength of the cheese matrix, leading to initiation and
213 propagation of cracks and slits within the cheese matrix (Prabin Lamichhane, Auty, Kelly, &
214 Sheehan, 2020). Overall, this suggests that strict control of cheese storage temperature may
215 contribute to minimizing and avoidance of development of slit and crack defects.

216 3.2. *Effect of salt content on solubility of CO₂*

217 The effect of three different salt concentrations on CO₂ solubility within the casein
218 matrices was studied (Fig. 2). Carbon dioxide solubility in the casein matrices decreased ($P =$
219 0.004) by ~22% with increasing average salt content from 0.04% (salt-in-moisture content:
220 0.06%, w/w) to 1.89% (salt-in-moisture content: 3.13%, w/w). This decrease in solubility
221 may be attributed to a salting-out effect on the solubility of CO₂ in the aqueous phase of the

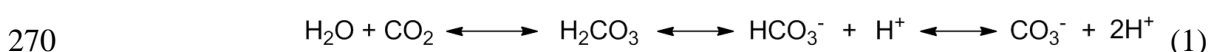
222 casein matrix by NaCl, as reported in previous studies (Acerbi et al., 2016; Carvalho, Pereira,
223 Gonçalves, Queimada, & Coutinho, 2015). In other words, salt is more polar than CO₂ and, in
224 solution, polar water molecules are more attracted to polar salt ions than nonpolar CO₂
225 molecules. Thus, fewer water molecules are available for dissolution of CO₂ in salt solutions,
226 resulting in reduced solubility of CO₂ (Holzammer, Finckenstein, Will, & Braeuer, 2016).
227 Moreover, increasing the salt concentration of aqueous solutions increases interfacial tension
228 providing another reason for a decrease in CO₂ solubility with increasing salt concentration
229 (Bachu & Bennion, 2009). Although the effect of salt on solubility behaviour of CO₂ in
230 aqueous solutions is well known, the role of NaCl on CO₂ solubility behaviour in solid food
231 matrices is less documented. Acerbi et al. (2016) studied the impact of salt content on CO₂
232 solubility behaviour in semi-hard cheese matrices and, in agreement with our results,
233 observed a significant decrease in CO₂ solubility (by ~25%) on increasing salt levels from 0.0
234 to 2.7 % (w/w).

235 The salt (or salt-in-moisture) concentration range selected was similar to those found
236 in eye-type cheeses and those involving propionic acid bacteria (PAB) fermentation cheeses
237 (Guinee, 2004; P. Lamichhane et al., 2019). In brine-salted cheese types, salt diffuses inward
238 from the rind to the centre of the cheese matrices, resulting in a decreasing salt gradient from
239 the rind to the centre (Guinee, 2004). The time for attainment of equilibrium in salt-in-
240 moisture content within the cheese matrix depends on composition, size and shape of the
241 cheese and ripening conditions, among other factors. It has been reported that Gouda (10 kg
242 wheel) and Emmental (60-130 kg wheel) cheese takes 7-9 weeks and > 4 months,
243 respectively, for attainment of equilibrium in salt-in-moisture content within the cheese
244 matrix (Daly, McSweeney, & Sheehan, 2010a; Guinee, 2004). Salt content in cheese can vary
245 from batch to batch due to manufacture-derived variables (e.g., temperature of brine and
246 brining time), or over a season due to variation in the composition of milk. Daly et al. (2010a)

247 observed a significantly higher salt content in the interior area of Emmental cheese blocks
 248 produced late in the season of manufacture than those produced early in the season. Such
 249 intra-cheese and inter-cheese variation in salt content will result in heterogeneity in the local
 250 concentration of CO₂ within or between cheese blocks, leading to variable internal pressure
 251 within or between cheeses. This may result in some areas of the cheese matrices or some
 252 batches of cheeses being more prone to the development of slits or cracks. Although no
 253 specific locations for development of slits and cracks within cheese matrices have been
 254 reported, a lower number of eyes have been found near the rind of Emmental cheese, where
 255 salt content is higher, than in the centre of the cheese (Bisig et al., 2019).

256 3.3. Effect of pH on solubility of CO₂

257 Solubility of CO₂ within the casein matrices increased ($P < 0.001$) by ~41% on
 258 increasing pH from 5.4 to 6.15 (Fig. 3) with a quadratic relationship ($R^2 = 0.93$) providing a
 259 better fit than a linear relationship ($R^2 = 0.84$). Interestingly, the solubility of CO₂ increased
 260 ($P = 0.026$) by ~14% on increasing pH from 5.4 to 5.8, whereas the solubility of CO₂
 261 increased ($P = 0.001$) by ~24% on increasing pH from 5.4 to 6.2. Such an increase in
 262 solubility is attributed to dissociation of an increasing fraction of the dissolved CO₂ as HCO₃⁻
 263 with increasing pH (Gill, 1988). In solution, CO₂ can exist as dissolved CO₂, carbonic acid,
 264 bicarbonate or carbonate ions, and the fraction of each form in solution depends on the pH of
 265 that solution (Equation 1). With increasing pH from 5.4 to 6.15, increasing quantities of
 266 carbonic acid will dissociate as bicarbonate ions (HCO₃⁻) and hydrogen ions (H⁺). As a result,
 267 higher quantities of CO₂ will dissolve in the aqueous phase of the casein matrix (Jakobsen &
 268 Bertelsen, 2002). At pH values below 8, the carbonate ions (CO₃⁻) in solution are present in
 269 negligible amounts (Dixon & Kell, 1989).



271 Very little is known regarding the effect of pH on CO₂ solubility in solid food systems
272 and no consensus have been found among the studies. Gill (1988), on investigating the effect
273 of pH of the muscle tissue of beef, pork and lamb, observed a linear increase in the solubility
274 of CO₂ with increasing pH from 5.4 to 6.9. Similarly, Jakobsen and Bertelsen (2006) reported
275 a slightly higher solubility of CO₂ in meat tissue with higher pH (5.83) than lower pH (5.66).
276 However, a difference of 0.5 pH units in the two meat types or fish types did not influence
277 the solubility of CO₂ (Sivertsvik & Jensen, 2005; Sivertsvik, Rosnes, & Jeksrud, 2004). This
278 discrepancy may be attributed to a comparison of data between different samples with
279 different compositions and possibly of different buffering capacity.

280 Dissolution of CO₂ in the aqueous phase of food matrices can decrease pH because of
281 formation of carbonic acid (Singh, Wani, Karim, & Langowski, 2012). There was a concern
282 that addition of CO₂ to the protein matrices would have resulted in a reduction in pH of the
283 samples, thus confounding our results relating to the effect of pH on CO₂ solubility.
284 Therefore, the pH of the samples was measured before and after packaging in a modified
285 atmosphere. It was observed that storage of small pieces (~10 g) of the casein matrices (3.1 %
286 salt-in-moisture, 60% moisture content, and 32% protein content) under a 100% CO₂
287 environment for 2 d reduced the pH by ~0.1 unit (data not shown). However, for water
288 samples of similar initial pH and salt-in-moisture content, pH decreased by ~2.3 units when
289 stored under 100% CO₂ environment for 2 d (data not shown). The comparatively small
290 decrease in pH of the casein matrices as compared to water may be attributed to its high
291 buffering capacity, as food matrices of higher buffering capacity are expected to exhibit a
292 greater resistance to pH change. Buffering capacity of food matrices largely depends on their
293 composition. Proteins, inorganic phosphate and organic acids are the main constituents
294 contributing to the buffering capacity of cheese (Salaün, Mietton, & Gaucheron, 2005).

295 During ripening, pH within the eye-type cheese matrices increases, due to the
296 proteolytic liberation of basic compounds and metabolism of lactic acid by propionic acid
297 bacteria, among other factors (P. Lamichhane, Kelly, et al., 2018a; Sheehan, Fenelon,
298 Wilkinson, & McSweeney, 2007). Studies have reported an increase in pH from 5.2-5.3 at 7-
299 11 d (before warm-room ripening) to ~5.5 at 35-41 d (after warm-room ripening) and 6.0-6.1
300 at 270 d of ripening in Swiss, Dutch and related eye-type cheeses (Govindasamy-Lucey,
301 Jaeggi, Martinelli, Johnson, & Lucey, 2011; P. Lamichhane, Kelly, et al., 2018a). Therefore,
302 an increase in pH, especially above pH 5.8, during ripening of cheese may contribute to an
303 increase in solubility of CO₂ within the cheese matrices. Moreover, natural cheese matrices
304 can have both macroscopic and microscopic pH gradients (Burdikova et al., 2015), which
305 may lead to heterogeneity in the concentration of CO₂ within the cheese matrices.

306 *3.4. Effect of moisture-to-protein ratio on CO₂ solubility*

307 To investigate the effect of moisture-to-protein ratio on CO₂ solubility, casein
308 matrices having different moisture and protein contents were prepared. Using approaches,
309 such as application of variable pressure during manufacture, freeze-drying, or incubation of
310 samples in various relative humidity environments, it was possible to obtain casein matrices
311 with moisture-to-protein ratios ranging between 0.03 and 2.5 (or moisture content range
312 between 2% and 67%).

313 Casein matrices with an average moisture-to-protein ratio of 0.027 ± 0.009
314 [corresponding to an average moisture and protein content of $2.10 \pm 0.67\%$ (w/w) and
315 $78.72 \pm 1.26\%$ (w/w) respectively] retained a considerable amount of CO₂, i.e., 161.7 ± 24.68
316 mmol kg⁻¹ atm⁻¹ at 5 °C (Fig. 4), in agreement with the results of Mitsuda, Kawa, Yamamoto,
317 and Nakajima (1975). Those authors observed that dried casein and gelatine powders retained
318 a considerable amount of CO₂ when stored under high CO₂ partial pressure. Although the
319 exact causes are not known, CO₂ adsorption by reactive sites in protein is considered as an

320 important factor (Cundari et al., 2009; Mitsuda et al., 1977). Polar and charged residues of
321 amino acids, such as ϵ -amino of lysine and guanidinium groups of arginine, are the potential
322 reactive sites in protein for adsorption of CO₂. Mitsuda et al. (1977) investigated the
323 reactivity of certain particular functional groups involved in CO₂ gas adsorption by protein.
324 Those authors obtained a good correlation between the amount of CO₂ adsorbed and the total
325 lysine and arginine content of protein samples and they concluded that the α -amino, ϵ -amino
326 and guanidinium groups are the preferred sites for adsorption of CO₂ by protein in the gas-
327 solid phase system. Cundari et al. (2009) analysed the binding of CO₂ to proteins utilizing a
328 combination of bioinformatics, molecular modelling, and first-principles quantum mechanics,
329 and concluded that the hydrogen bonds between the functional groups of the amino acids and
330 the oxygen sites on the carbon dioxide were involved in the CO₂ adsorption process.

331 The relationship between moisture-to-protein ratio and CO₂ solubility was non-linear
332 (Fig. 4), and can be divided into three distinct regions: (1) a rapid decrease in CO₂ solubility
333 on increasing the moisture-to-protein ratio from ~ 0.03 to ~ 0.5 ; (2) a relatively slower
334 decrease in CO₂ solubility with increasing moisture-to-protein ratio from ~ 0.5 to ~ 1.7 ; and
335 (3) a small but significant increase in CO₂ solubility on increasing moisture-to-protein ratio
336 from ~ 1.7 to ~ 2.5 . Around a 4-fold decrease in CO₂ solubility was observed when the
337 average moisture-to-protein ratio of casein matrices increased from ~ 0.03 to 0.5 . Mitsuda et
338 al. (1975) also reported similar solubility behaviour of CO₂ in dried casein and gelatine
339 powder as a function of moisture content. The solubility of CO₂ in casein or gelatine powder
340 decreased by $>90\%$ when their moisture content increased from ~ 5 or 10% (w/w) to 20 or
341 40% (w/w). The authors also observed a rapid decrease in CO₂ solubility of casein or gelatine
342 powder prior to a gradual decrease in their CO₂ solubility on increasing moisture levels. Pre-
343 adsorbed water may interact with the reactive sites of casein matrices making those reactive
344 sites unavailable for interaction with CO₂, thus decreasing the CO₂ adsorption/solubilisation

345 capacity of hydrated casein matrices. A similar moisture-dependent CO₂ adsorption
346 behaviour has also been observed in other non-food materials. For example, Ozdemir and
347 Schroeder (2009) observed a lower CO₂ adsorption capacity of wet coals compared to that of
348 dried coals. Those authors speculated that the adsorbed water occupies the pore spaces or the
349 active sites for the adsorption of CO₂.

350 Water in casein matrices is either present in a bulk (freezable at – 40 °C) or bound
351 form (non-freezable at – 40 °C) (P. Lamichhane, Kelly, & Sheehan, 2018b; McMahon et al.,
352 1999), and the latter is typically considered to be so-called primary hydration water and
353 primarily related to the solvation of polar and charged residues (Huppertz et al., 2017). In
354 casein matrices of moisture-to-protein ratio up to 0.5, almost all moisture (> 98% of total
355 moisture) was found to be in non-freezable form (Fig. 4). This result further supports the
356 hypothesis that the pre-adsorbed water may interact with the reactive sites (e.g., polar and
357 charged residues) of casein matrices, making those reactive sites unavailable for CO₂
358 interaction.

359 The solubility of CO₂ in casein matrices first decreased ($P < 0.05$) by ~23% with
360 increasing moisture-to-protein ratio from ~0.5 to ~1.7 and then increased ($P < 0.05$) by ~21%
361 with increasing moisture-to-protein ratio from ~1.7 to ~2.5 (Fig. 4, inset). Such complex
362 relationships observed between CO₂ solubility and moisture-to-protein ratio may be attributed
363 to interactive effects of moisture and protein content on CO₂ solubility. This suggests that
364 both water and protein components of casein matrices have an important role on CO₂
365 solubility.

366 In eye-type cheeses, the moisture-to-protein ratio is between 1.2 and 2.0, e.g., ~1.8 in
367 Maasdam (P. Lamichhane, Kelly, et al., 2018a; P. Lamichhane, Pietrzyk, et al., 2018), ~1.7 in
368 Edam and ~1.25 in Emmental (Deegan et al., 2013). Therefore, the solubility of CO₂ studied
369 in casein matrices with a protein-to-moisture ratio between 1.0 and 2.0 are particularly

370 important for hard and semi-hard eye-type cheeses. Moisture-to-protein ratios in cheese may
371 also vary on a batch-to-batch basis, due to seasonal variations in the composition of milk and
372 thus the resultant cheeses. Therefore, these results could form the basis for development of a
373 robust model for prediction of CO₂ solubility in a wide variety of cheeses of different
374 compositions, as models reported in previous studies were limited to the cheese type under
375 study (Acerbi et al., 2016; Jakobsen et al., 2009).

376 Although the solubility behaviour of CO₂ studied in casein matrices with a protein-to-
377 moisture ratio below 1.0 is not relevant to natural cheese matrices, such knowledge may be
378 useful when designing modified atmosphere packaging for cheese powders, where the protein
379 to moisture ratio in spray-dried cheese powders was reported to vary between 0.05 and 0.12
380 (Felix da Silva, Larsen, Hougaard, & Ipsen, 2017).

381 Various approaches used in this study to achieve different moisture-to-protein ratios
382 could potentially lead to differences in microstructure of the casein matrices. However,
383 microstructure seems to have a low influence on the solubility of CO₂. For example, Jakobsen
384 et al. (2006) did not observe a significant difference in the amount of CO₂ adsorbed between
385 meat samples (i.e., whole versus minced meat) having similar compositions but different
386 microstructures. Further research is recommended to elucidate the influence of microstructure
387 on the solubility of CO₂.

388 **4. Conclusions**

389 This study investigated the solubility behaviour of CO₂ in casein matrices,
390 representing varying conditions of the protein-water phase of semi-hard cheese matrices.
391 Both compositional (i.e, moisture-to-protein ratio and salt-in-moisture content) and ripening-
392 related (i.e., pH and temperature) parameters had a significant influence on CO₂ solubility of
393 casein matrices. Variation in the cheese composition from batch to batch due to differences in

394 milk composition or manufacture derived variables, such as time of the day of manufacture,
395 plant temperature, temperature of brine and brining times, and rennet-to-casein ratio, may
396 result in certain batches being more at risk for development of slits and cracks.

397 Overall, the result obtained from this study should form the basis for development of
398 a robust model for CO₂ solubility in a wide variety of cheese types or where the composition
399 of cheese may vary within a commercial cheese production plant. Such knowledge may help
400 to improve the quality and consistency of eye-type cheese by minimizing or avoiding
401 development of slits and cracks.

402 **Acknowledgements**

403 This study was funded by the Dairy Levy Trust Fund (RMIS 6259) administered by Dairy
404 Research Ireland, and in part by Ornuu (Dubin, Ireland). Prabin Lamichhane was in receipt of
405 a Teagasc Walsh Fellowship; he also received 'Teagasc International Training Award, 2019'
406 to conduct this study in collaboration with the University of Copenhagen. The authors
407 acknowledge Henriette Rifbjerg Erichsen (University of Copenhagen) for technical assistance
408 with CO₂ solubility determination.

409 **References**

- 410 Acerbi, F., Guillard, V., Guillaume, C., & Gontard, N. (2016). Impact of selected
 411 composition and ripening conditions on CO₂ solubility in semi-hard cheese. *Food*
 412 *Chemistry*, *192*, 805-812. <https://doi.org/10.1016/j.foodchem.2015.07.049>.
- 413 Adhikari, B. M., Truong, T., Bansal, N., & Bhandari, B. (2018). Use of gases in dairy
 414 manufacturing: A review. *Critical Reviews in Food Science and Nutrition*, *58*, 2557-
 415 2569. <https://doi.org/10.1080/10408398.2017.1333488>.
- 416 Bachu, S., & Bennion, D. B. (2009). Interfacial tension between CO₂, freshwater, and brine
 417 in the range of pressure from (2 to 27) MPa, temperature from (20 to 125) °C, and
 418 water salinity from (0 to 334 000) mg·L⁻¹. *Journal of Chemical and Engineering*
 419 *Data*, *54*, 765-775. <https://doi.org/10.1021/jc800529x>.
- 420 Bisig, W., Guggisberg, D., Jakob, E., Turgay, M., Irmeler, S., Wechsler, D., & Fröhlich-
 421 Wyder, M.-T. (2019). The effect of NaCl and metabolic profile of propionibacteria on
 422 eye formation in experimental Swiss-type cheese. *International Dairy Journal*, *89*,
 423 86-95. <https://doi.org/10.1016/j.idairyj.2018.09.001>.
- 424 Burdikova, Z., Svindrych, Z., Pala, J., Hickey, C. D., Wilkinson, M. G., Panek, J., Auty, M.
 425 A. E., Periasamy, A., & Sheehan, J. J. (2015). Measurement of pH micro-
 426 heterogeneity in natural cheese matrices by fluorescence lifetime imaging. *Frontiers*
 427 *in microbiology*, *6*, 183. <https://doi.org/10.3389/fmicb.2015.00183>.
- 428 Carvalho, P. J., Pereira, L. M. C., Gonçalves, N. P. F., Queimada, A. J., & Coutinho, J. A. P.
 429 (2015). Carbon dioxide solubility in aqueous solutions of NaCl: Measurements and
 430 modeling with electrolyte equations of state. *Fluid Phase Equilibria*, *388*, 100-106.
 431 <https://doi.org/10.1016/j.fluid.2014.12.043>.
- 432 Coffie-Agblor, R., Muir, W. E., Sinicio, R., Cenkowski, S., & Jayas, D. S. (1995).
 433 Characteristics of carbon dioxide sorption by stored wheat. *Journal of Stored*
 434 *Products Research*, *31*, 317-324. [https://doi.org/10.1016/0022-474X\(95\)00022-Y](https://doi.org/10.1016/0022-474X(95)00022-Y).
- 435 Cundari, T. R., Wilson, A. K., Drummond, M. L., Gonzalez, H. E., Jorgensen, K. R., Payne,
 436 S., Braunfeld, J., De Jesus, M., & Johnson, V. M. (2009). CO₂-formatics: How do
 437 proteins bind carbon dioxide? *Journal of Chemical Information and Modeling*, *49*,
 438 2111-2115. <https://doi.org/10.1021/ci9002377>.
- 439 Daly, D. F. M., McSweeney, P. L. H., & Sheehan, J. J. (2010a). Investigation of underlying
 440 factors in split and secondary fermentation defects in rindless block Swiss-type
 441 cheeses. *Australian Journal of Dairy Technology*, *65*, 214-216.
- 442 Daly, D. F. M., McSweeney, P. L. H., & Sheehan, J. J. (2010b). Split defect and secondary
 443 fermentation in Swiss-type cheeses – A review. *Dairy Science & Technology*, *90*, 3-
 444 26. <https://doi.org/10.1051/dst/2009036>.
- 445 Deegan, K. C., Heikintalo, N., Ritvanen, T., Putkonen, T., Rekonen, J., McSweeney, P. L. H.,
 446 Alatossava, T., & Tuorila, H. (2013). Effects of low-pressure homogenisation on the
 447 sensory and chemical properties of Emmental cheese. *Innovative Food Science &*
 448 *Emerging Technologies*, *19*, 104-114. <https://doi.org/10.1016/j.ifset.2013.04.008>.
- 449 Dixon, N. M., & Kell, D. B. (1989). The control and measurement of 'CO₂' during
 450 fermentations. *Journal of Microbiological Methods*, *10*, 155-176.
 451 [https://doi.org/10.1016/0167-7012\(89\)90048-1](https://doi.org/10.1016/0167-7012(89)90048-1).
- 452 Felix da Silva, D., Larsen, F. H., Hougaard, A. B., & Ipsen, R. (2017). The influence of raw
 453 material, added emulsifying salt and spray drying on cheese powder structure and
 454 hydration properties. *International Dairy Journal*, *74*, 27-38.
 455 <https://doi.org/10.1016/j.idairyj.2017.01.005>.
- 456 Gill, C. O. (1988). The solubility of carbon dioxide in meat. *Meat Science*, *22*, 65-71.
 457 [https://doi.org/10.1016/0309-1740\(88\)90027-7](https://doi.org/10.1016/0309-1740(88)90027-7).

- 458 Govindasamy-Lucey, S., Jaeggi, J., Martinelli, C., Johnson, M., & Lucey, J. (2011).
459 Standardization of milk using cold ultrafiltration retentates for the manufacture of
460 Swiss cheese: Effect of altering coagulation conditions on yield and cheese quality.
461 *Journal of Dairy Science*, *94*, 2719-2730. <https://doi.org/10.3168/jds.2010-3842>.
- 462 Guinee, T. P. (2004). Salting and the role of salt in cheese. *International Journal of Dairy*
463 *Technology*, *57*, 99-109. <https://doi.org/10.1111/j.1471-0307.2004.00145.x>.
- 464 Holzammer, C., Finckenstein, A., Will, S., & Braeuer, A. S. (2016). How sodium chloride
465 salt inhibits the formation of CO₂ gas hydrates. *The Journal of Physical Chemistry B*,
466 *120*, 2452-2459. <https://doi.org/10.1021/acs.jpcc.5b12487>.
- 467 Huppertz, T., Gazi, I., Luyten, H., Nieuwenhuijse, H., Alting, A., & Schokker, E. (2017).
468 Hydration of casein micelles and caseinates: Implications for casein micelle structure.
469 *International Dairy Journal*, *74*, 1-11. <https://doi.org/10.1016/j.idairyj.2017.03.006>.
- 470 IDF. (1996). Milk. Determination of fat content (Röse Gottlieb gravimetric method).
471 International Dairy Federation, Brussels, Belgium.
- 472 Jakobsen, M., & Bertelsen, G. (2002). The use of CO₂ in packaging of fresh red meats and its
473 effect on chemical quality changes in the meat: A review. *Journal of Muscle Foods*,
474 *13*, 143-168. <https://doi.org/10.1111/j.1745-4573.2002.tb00326.x>.
- 475 Jakobsen, M., & Bertelsen, G. (2006). Solubility of carbon dioxide in fat and muscle tissue.
476 *Journal of Muscle Foods*, *17*, 9-19. <https://doi.org/10.1111/j.1745-4573.2006.00029.x>.
- 477
478 Jakobsen, M., Jensen, P. N., & Risbo, J. (2009). Assessment of carbon dioxide solubility
479 coefficients for semihard cheeses: the effect of temperature and fat content. *European*
480 *Food Research and Technology*, *229*, 287-294. <https://doi.org/10.1007/s00217-009-1059-3>.
- 481
482 Lamichhane, P. (2019). *Influence of milk pre-treatment, and changes in cheese structure and*
483 *carbon dioxide solubility on the development of split defects in cheese*. PhD thesis,
484 University College Cork.
- 485 Lamichhane, P., Auty, M. A. E., Kelly, A. L., & Sheehan, J. J. (2020). Dynamic in situ
486 imaging of semi-hard cheese microstructure under large-strain tensile deformation:
487 Understanding structure-fracture relationships. *International Dairy Journal*, *103*,
488 104626. <https://doi.org/10.1016/j.idairyj.2019.104626>.
- 489 Lamichhane, P., Kelly, A. L., & Sheehan, J. J. (2018a). Effect of milk centrifugation and
490 incorporation of high-heat-treated centrifugate on the composition, texture, and
491 ripening characteristics of Maasdam cheese. *Journal of Dairy Science*, *101*, 5724-
492 5737. <https://doi.org/10.3168/jds.2017-14178>.
- 493 Lamichhane, P., Kelly, A. L., & Sheehan, J. J. (2018b). Symposium review: Structure-
494 function relationships in cheese. *Journal of Dairy Science*, *101*, 2692-2709.
495 <https://doi.org/10.3168/jds.2017-13386>.
- 496 Lamichhane, P., Pietrzyk, A., Feehily, C., Cotter, P. D., Mannion, D. T., Kilcawley, K. N.,
497 Kelly, A. L., & Sheehan, J. J. (2018). Effect of milk centrifugation and incorporation
498 of high heat-treated centrifugate on the microbial composition and levels of volatile
499 organic compounds of Maasdam cheese. *Journal of Dairy Science*, *101*, 5738-5750.
500 <https://doi.org/10.3168/jds.2017-14180>.
- 501 Lamichhane, P., Sharma, P., Kennedy, D., Kelly, A. L., & Sheehan, J. J. (2019).
502 Microstructure and fracture properties of semi-hard cheese: Differentiating the effects
503 of primary proteolysis and calcium solubilization. *Food Research International*, *125*,
504 108525. <https://doi.org/10.1016/j.foodres.2019.108525>.
- 505 McMahan, D. J., Fife, R. L., & Oberg, C. J. (1999). Water partitioning in Mozzarella cheese
506 and its relationship to cheese meltability. *Journal of Dairy Science*, *82*, 1361-1369.
507 [https://doi.org/10.3168/jds.S0022-0302\(99\)75361-0](https://doi.org/10.3168/jds.S0022-0302(99)75361-0).

- 508 Mitsuda, H., Kawa, F., Yamamoto, A., & Nakajima, K. (1975). Carbon dioxide-protein
509 interaction in a gas-solid phase. *Journal of Nutritional Science and Vitaminology*, 21,
510 151-162. <https://doi.org/10.3177/jnsv.21.151>.
- 511 Mitsuda, H., Kawai, F., Yamamoto, A., Suzuki, F., Nakajima, K., & Yasumoto, K. (1977).
512 Identification and properties of reactive sites in protein capable of binding carbon
513 dioxide in a gas-solid phase system. *Journal of Nutritional Science and Vitaminology*,
514 23, 145-152. <https://doi.org/10.3177/jnsv.23.145>.
- 515 Ozdemir, E., & Schroeder, K. (2009). Effect of moisture on adsorption isotherms and
516 adsorption capacities of CO₂ on coals. *Energy & Fuels*, 23, 2821-2831.
517 <https://doi.org/10.1021/ef801126a>.
- 518 Salaün, F., Mietton, B., & Gaucheron, F. (2005). Buffering capacity of dairy products.
519 *International Dairy Journal*, 15, 95-109.
520 <https://doi.org/10.1016/j.idairyj.2004.06.007>.
- 521 Sheehan, J. J., Fenelon, M. A., Wilkinson, M. G., & McSweeney, P. L. H. (2007). Effect of
522 cook temperature on starter and non-starter lactic acid bacteria viability, cheese
523 composition and ripening indices of a semi-hard cheese manufactured using
524 thermophilic cultures. *International Dairy Journal*, 17, 704-716.
525 <https://doi.org/10.1016/j.idairyj.2006.08.011>.
- 526 Singh, P., Wani, A. A., Karim, A. A., & Langowski, H.-C. (2012). The use of carbon dioxide
527 in the processing and packaging of milk and dairy products: A review. *International*
528 *Journal of Dairy Technology*, 65, 161-177. [https://doi.org/10.1111/j.1471-](https://doi.org/10.1111/j.1471-0307.2011.00744.x)
529 [0307.2011.00744.x](https://doi.org/10.1111/j.1471-0307.2011.00744.x).
- 530 Sivertsvik, M., & Jensen, J. S. (2005). Solubility and absorption rate of carbon dioxide into
531 non-respiring foods. Part 3: Cooked meat products. *Journal of Food Engineering*, 70,
532 499-505. <https://doi.org/10.1016/j.jfoodeng.2004.10.005>.
- 533 Sivertsvik, M., Rosnes, J. T., & Jeksrud, W. K. (2004). Solubility and absorption rate of
534 carbon dioxide into non-respiring foods. Part 2: Raw fish fillets. *Journal of Food*
535 *Engineering*, 63, 451-458. <https://doi.org/10.1016/j.jfoodeng.2003.09.004>.
- 536 Truong, T., Palmer, M., Bansal, N., & Bhandari, B. (2017). Investigation of solubility of
537 carbon dioxide in anhydrous milk fat by lab-scale manometric method. *Food*
538 *Chemistry*, 237, 667-676. <https://doi.org/10.1016/j.foodchem.2017.05.141>.
- 539 Walstra, P., Wouters, J. T., & Geurts, T. J. (2005). *Dairy Science and Technology*. Boca
540 Raton, FL, USA: CRC press.

542

543

544 **Figure legends**

545 **Fig. 1.** (a) Carbon dioxide concentration in casein matrices as a function of carbon dioxide
 546 partial pressure in the headspace of modified atmosphere packages and temperature; (\circ) 5 °C,
 547 (\square) 12 °C, and (\diamond) 25 °C; error bars represent standard deviations of means ($n = 3$). (b)
 548 Carbon dioxide concentration in the casein matrices as a function of temperature; (\blacktriangle), mean
 549 of data from three replicate experiments; (\bullet), individual data point; P represents P-value;
 550 error bars represent standard deviations of means ($n = 3$). Average composition (\pm standard
 551 deviation) of samples: moisture content = 61.43 ± 1.32 (% w/w), protein content = 31.20 ± 0.05
 552 (% w/w), fat content: 0.39 ± 0.10 (% w/w), salt content = 1.90 ± 0.05 (% w/w), salt-in-
 553 moisture content = 3.09 ± 0.05 (% w/w), and pH = 5.84 ± 0.02 .

554 **Fig. 2.** Effect of salt-in-moisture content on the solubility of carbon dioxide in casein
 555 matrices; (\blacktriangle), mean of data from three replicate experiments; (\bullet), individual data point; P
 556 represent P-value. Error bars represent standard deviations of means ($n = 3$). Average
 557 composition (\pm standard deviation) of samples: moisture content = 61.38 ± 1.30 (% w/w),
 558 protein content = 31.11 ± 1.78 (% w/w), fat content = 0.39 ± 0.08 (% w/w), and pH =
 559 5.37 ± 0.03 .

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

566 **Fig. 4.** Relationships between moisture-to-protein ratio and (\blacktriangle) CO₂ solubility or (\blacksquare) non-
 567 freezable moisture (% of the total moisture) in renneted-casein matrices; (\bullet) individual data
 568 point. Inset: magnification of CO₂ solubility data for casein matrices of moisture-to-protein

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

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

573 **Prateek Sharma**: Methodology.

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

575 **Jens Risbo**: Methodology, Conceptualization, Resources, Supervision.

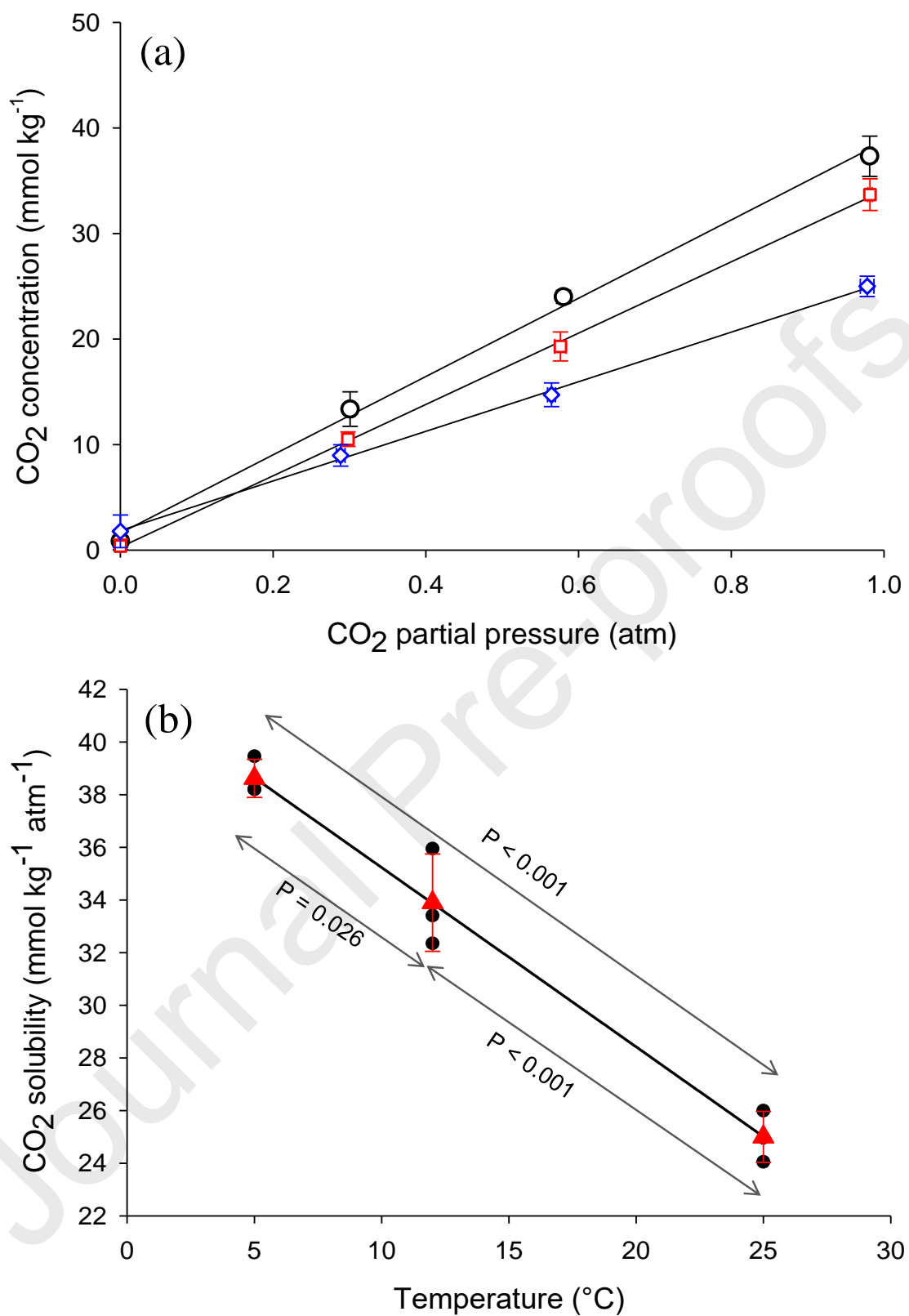
576 **Fergal P. Rattray**: Conceptualization, Resources, Supervision.

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

579

580 Declaration of Interest Statement: None

581

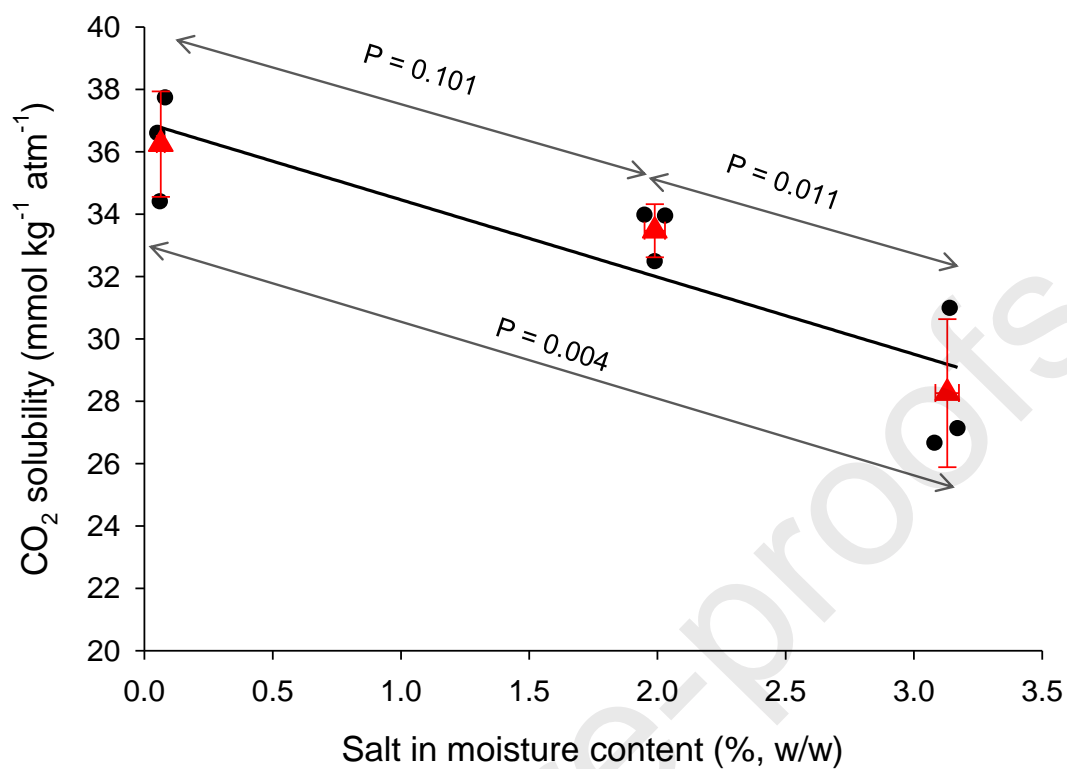


582

583 Fig. 1

584

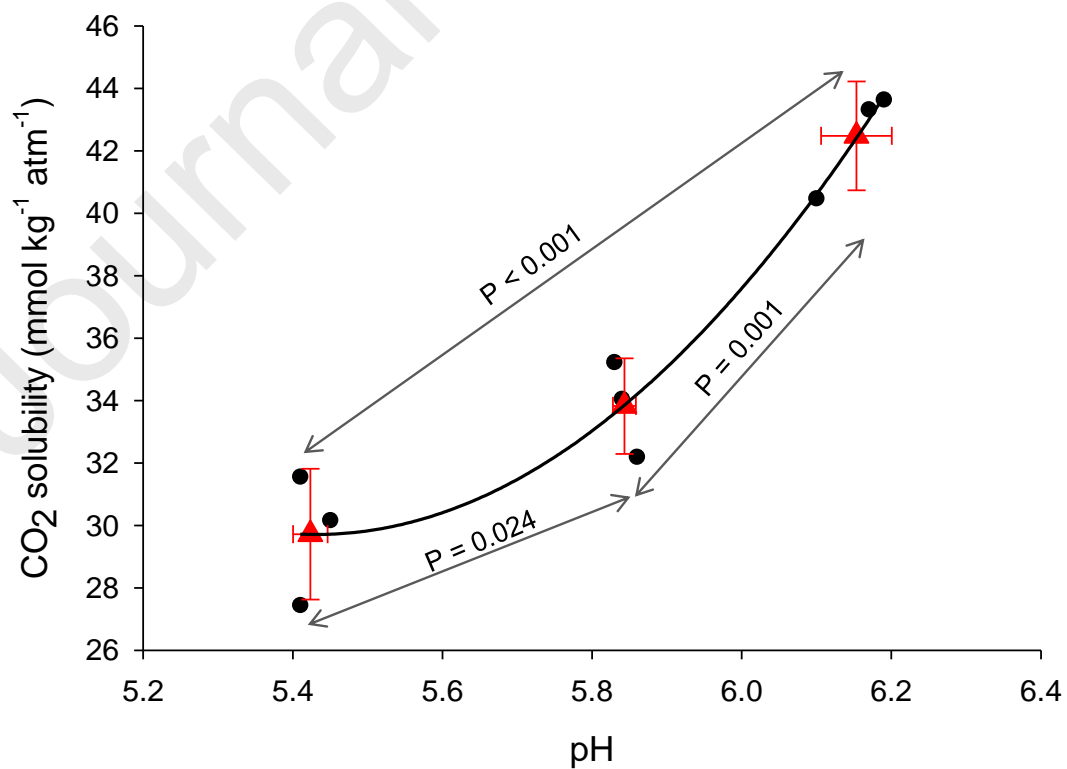
585



586

587 Fig. 2.

588

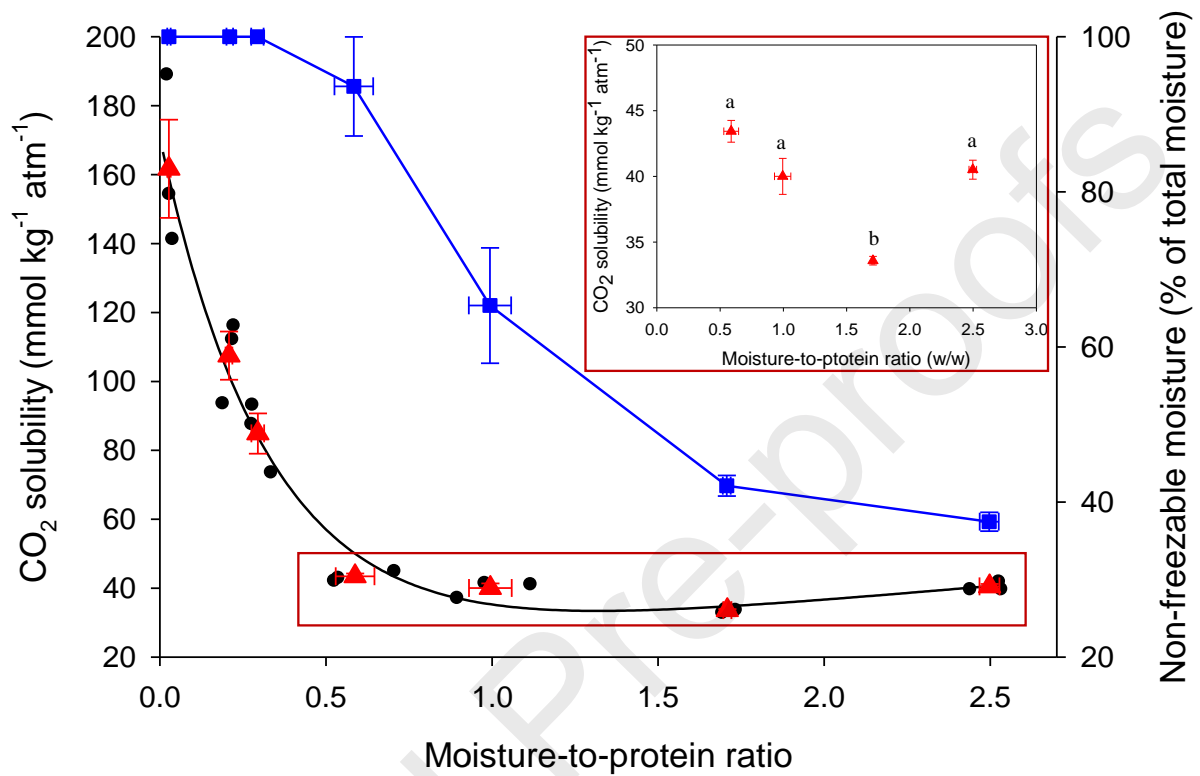


589

590 Fig. 3.

591

592



593

594 Fig. 4.

595

596

597 Highlights

- 598 • Model casein matrices with varying moisture-to-protein ratio, pH, and salt content
599 were prepared
- 600 • The CO₂ solubility of samples decreased with increasing temperature and salt-in-
601 moisture content
- 602 • A non-linear relationship was observed between CO₂ solubility and the moisture-to-
603 protein ratio

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

606

607

Journal Pre-proofs