Using ^{1}H and ^{23}Na NMR relaxometry as a novel tool to monitor the moisture and salt distribution in commercial low-moisture part skim mozzarella

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Abstract

The zonal variations in ^{1}H or ^{23}Na content in commercial low-moisture part-skim mozzarella with a uniform salt distribution, obtained by dry-salting, or with a salt gradient, obtained by dry-salting and brining, were profiled using a combined ^{1}H and ^{23}Na NMR relaxometry procedure.

1D concentration-distance profiles were successfully constructed in all cheeses from the surface to the core. The proposed method enabled rapid (~ 21 min) distinction between dry-salted and brined cheese in a non-invasive way. The uptake of salt in cheese brined for 0 to 24 h was determined, and the derived effective diffusion coefficient of ^{23}Na ($3.23 \bullet 10^{-10}$ m^2 s^{-1}) compared favorably with values reported for other semi-hard cheeses during brining, whereas the ^{23}Na concentration-distance profiles of brined cheese stored for 2 to 44 d at 4 °C corresponded to an effective diffusion coefficient of about $5.21 \bullet 10^{-10}$ m^2 s^{-1}. Small variations in moisture content in the cheese could not be distinguished from the random fluctuations in ^{1}H signal intensity. The findings indicated that the proposed approach is a promising tool to evaluate salt distribution profiles in cheese.

1. Introduction

Salt is dosed during the manufacturing of low-moisture part-skim (LMPS) mozzarella to limit the growth of spoilage micro-organisms and to impart the cheese with flavor. This process is done either by dry-salting the hot fermented curd (~ 60 °C) with subsequent kneading, mixing and molding, or by submerging the molded cheese blocks in cold brine (~ 20 %, w/w NaCl; 4 °C) which helps to rapidly lower the temperature of the hot cheese to 4 °C (Fox, Guinee, Cogan, & McSweeney, 2017; Kindstedt, 2001). The former results in cheese with a uniform salt distribution, whereas the latter deposits salt at the surface of the cheese, which gradually diffuses into the core during storage (Guinee, 2004). Moreover, the osmotic pressure gradient during brining induces the migration of cheese moisture from the core to the surface of the submerged cheese blocks, and lowers their overall moisture content (Fox et al., 2017; Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999).

Cross-sectional studies suggest that the salt-in-moisture (S/M) affects the hydration of the protein (Guinee, 2004; Guo, Gilmore, & Kindstedt, 1997; Kindstedt, Kiely, & Gilmore, 1992;) and the solubilization of colloidal Ca (through displacement of Ca with Na) (Ah & Tagalpallewar, 2017; Gobet et al., 2010; Smith, Carr, Golding, & Reid, 2018), which in turn have been reported as critical mediators determining the cheese functionality (Guinee, Feeney, Auty, & Fox, 2002; McMahon & Oberg, 2017). Hence, the presence of a salt gradient during brining may increase the zonal variability in cheese functional properties (Guinee, 2004; Kindstedt et al., 1992). As the functional properties of LMPS mozzarella are central to its application as a pizza topping, there is a need to examine the spatio-temporal evolution of the salt gradient during and after brining, as well as the relation between this salt gradient and the zonal variability in cheese functionality.

Kindstedt et al. (1992) examined the effects of regional variations in commercial brine-salted LMPS mozzarella on its apparent viscosity and free oil formation after heating. The authors found

54 that the core of the cheese had a lower apparent viscosity and a higher tendency to oil off than the
55 surface. It was suggested that the higher NaCl content at the surface improved the emulsifying
56 capacity of the casein through displacement of Ca^{2+} with Na^{+}, and thus increased the level of free
57 casein available to emulsify fat. It is worth noting, however, that the core of the cheese also
58 contained more moisture than the surface, and that this moisture gradient varied greatly between
59 brine-salted LMPS mozzarella sampled from different commercial plants (Kindstedt, 2001;
60 Kindstedt et al., 1996), possibly due to fluctuating thermal gradients in the hot cheese blocks after
61 submersion into cold brine (Kindstedt, Larose, Gilmore, & Davis, 1996; Walstra et al., 1999).
62 Standard methods to determine the moisture or salt content in cheese rely on grating the cheese and
63 subsequently determining the dry weight of the sample or the chloride ion concentration through
64 potentiometric or colorimetric titration with $AgNO_3$, respectively. Applying these methods to
65 evaluate the moisture or salt gradient in brine-salted cheese is time-laborious, and has a limited
66 spatial resolution, providing an average value for the grated sample.
67 Nuclear magnetic resonance (NMR) is a non-invasive and non-destructive technique that requires
68 no sample pre-treatment, provides insights in the distribution and compartmentalization of food
69 constituents, and is fast becoming a key instrument in food analytics (Blümich & Singh, 2018;
70 Hatzakis, 2019; Kirtil & Oztop, 2016). A number of NMR techniques (^{1}H NMR spectroscopy,
71 diffusometry and relaxometry) have been developed to evaluate the major components in
72 mozzarella cheese, most notably moisture and fat (Gianferri, D'Aiuto, Curini, Delfini, & Brosio,
73 2007; Kuo, Gunasekaran, Johnson, & Chen, 2001; Smith, Hindmarsh, Carr, Golding, & Reid, 2017;
74 Vermeir, Declerck, To, Kerkaert, & Van der Meeren, 2019). In addition, the sodium ion
75 concentration was successfully quantified in pilot-scale produced semi-hard cheese using single-
76 quantum ^{23}Na NMR spectroscopy (Gobet et al., 2010).

77 The objectives of the current study were two-fold. First, we introduce the use of ^{1}H NMR and ^{23}Na
78 NMR as a novel tool to evaluate zonal variations in moisture or salt content in industrial LMPS
79 mozzarella by obtaining 1-D scans of the ^{1}H and ^{23}Na distribution. Secondly, the potential of the
80 proposed method to distinguish between cheeses with different zonal variations in moisture or salt
81 content were evaluated. The latter cheeses were produced by varying the salting method, the
82 brining time, or the storage time at 4 °C.

2. Materials and methods

2.1. Control cheese

Commercial LMPS mozzarella was supplied by Milcobel CV (Langemark, Belgium). The target compositional values for dry matter, fat, protein and NaCl are about 53, 42, 25, and 1.3 %, respectively. The target NaCl content is attained by first dry-salting the curd to about 0.9 %, w/w, and subsequently by molding the curd into blocks (2.5 kg; 28 cm x 10 cm x 8 cm) and submerging the molded blocks into brine of about 20 %, w/w NaCl at 4 °C for 6 to 8 h. After brining, the cheese blocks were rinsed with water, vacuum-packed and stored at 4 °C. Cheese produced via this protocol is denoted as the control.

2.2. Cheese composition

Grated LMPS mozzarella was analyzed for fat and protein via near infrared spectroscopy using a FoodScan^{TM} for dairy (FOSS, Hillerød, Denmark), and for moisture and NaCl using standard International Dairy Federation methods (IDF 2004, 2006). The NaCl of the cheese was expressed as a percentage of total cheese mass (%, w/w), and as a percentage of total cheese moisture (S/M; %, w/w).

2.3. NMR relaxometry

First, the instrument settings of the NMR setup for ^{1}H- and ^{23}Na-relaxometry were optimized.

2.3.1. Instrument settings

The ^{1}H- and ^{23}Na-relaxometry measurements were conducted using a custom-built NMR setup at the University of Technology, Eindhoven operating at a magnetic field of 0.8 T, corresponding to 33.960 MHz for ^{1}H and 8.983 MHz for ^{23}Na. A Hahn spin echo pulse sequence was employed using a 90° pulse (^{1}H: 30 µs; ^{23}Na: 35 µs) and a 180° pulse (^{1}H: 60 µs; ^{23}Na: 70 µs) at a constant

magnetic field gradient (0.3 T m^{-1}). The echo times used in these measurements for ^{1}H- and ^{23}Na were 250 and 400 µs, respectively. At these echo times, no signal is measured from solids like NaCl crystals. This NMR setup is equipped with a Faraday shield as to reduce the influence of variations of the tuning due to changes in the sample, hence, making the measurements quantitative.

2.3.1.1. Repetition time

Preliminary experiments using $CH_3COONa.3H_2O$ salt solutions (2.3, 4.7, 7.0, 9.3 and 11.6 %, w/w) were conducted to determine the repetition time. Sodium acetate was chosen instead of NaCl to simultaneously measure ^{1}H-signals of protons present in forms other than H_2O which was better representative for the protons in cheese as the latter interact with the calcium-phosphate para-casein network to various degrees (Vermeir et al., 2019). Each $CH_3COONa.3H_2O$ solution was prepared by weighing $CH_3COONa.3H_2O$ in a beaker to which distilled water was added to 52 g. The density of each solution was measured at 20 °C using a DMA 5000M (Anton Paar, Graz, Austria) (Table 1). Based on these measurements the repetition time was set at 1000 or 50 ms respectively for ^{1}H- and ^{23}Na, which was approximately equal to 5 times the T_1 relaxation time.

2.3.1.2. Number of scans

The average signal intensity at the core or surface of control cheese (stored for 51 d at 4 °C) was measured by taking 2, 4, 8, 16, 32, 64, 128 or 256 scans at each location. The signal-to-noise ratio at the core or surface of the cheese was compared with those of air (background noise) and two salt solutions (2.3 and 11.6 %, w/w $CH_3COONa.3H_2O$). The number of scans was set at 128 which corresponded to a minimal signal-to-noise ratio of about 5 and a measuring time of 21 min per sample.

2.3.2. Sample preparation for ^{1}H- and ^{23}Na-profile analysis

Polyethylene terephthalate (PET) open tubes (1200 mm length, 25.8 mm inner diameter, 0.6 mm wall thickness, ClearTec) were cut to a length of 140 mm. The edge at one side was sharpened to facilitate the sampling procedure. The sharpened edge of the PET tube was gently pushed vertically into the central part of the cheese block to yield cylindrical mozzarella samples of ~24 mm diameter, and ~80 mm length. The procedure was manually carried out at a rate of approximately 80 mm min^{-1} and with a 70 mm intertube spacing in order to minimize sample deformation. Using this method, the diameter of the obtained cylindrical samples at the bottom, center and top regions were (23.8 ± 1.0), (23.9 ± 0.7), and (24.4 ± 0.4) mm, respectively (n = 29), suggesting that a slightly wider thickness at the top of the cylindrical sample could not be avoided. The tube was closed at both sides using custom-made polyvinylchloride plugs. In order to measure the distribution, the cylindrical cheese samples were gradually moved through the NMR setup using a stepper motor. The first measurements were taken at the bottom of the sample, after which the stepper motor raised the sample sequentially by 2.54 mm before any new measurement to obtain a complete ^{1}H- and ^{23}Na-profile of the sample. In order to minimize temperature gradients, each sample was prepared within 5 min after removing the cheese from cold storage.

The signal intensities obtained from ^{1}H-relaxometry measurements at the different sample locations (i) were reported as normalized values by dividing each value ($I_{H,i}$) by the maximum signal intensity (I_{H_max}).

The signal intensities obtained from ^{23}Na-relaxometry measurements ($I_{Na,i}$) were corrected by dividing each signal intensity value by its corresponding normalized ^{1}H signal intensity value ($I_{H,i}/I_{H\_max}$). The correction on the ^{23}Na signal intensity value was done to accommodate for sample deformation which may arise from the sampling procedure or from fluctuations in sample temperature.

154 2.3.3. Examining the repeatability of cheese ^{1}H- and ^{23}Na-profile analysis

155 Control cheese was stored for about 1 month at 4 °C to allow the moisture and salt distribution to
156 equilibrate. The ^{1}H- and ^{23}Na-profile of this cheese was measuring 10 times in succession to
157 examine the repeatability of the method, and to investigate possible variations induced by a
158 variability in sample temperature.

159

160 2.4. Using ^{1}H- and ^{23}Na-relaxometry to distinguish between cheeses with different zonal variations
161 in moisture or salt content

162 After defining the instrument settings and examining the repeatability of the proposed method, the
163 potential of ^{1}H- and ^{23}Na-NMR relaxometry to identify differences between cheeses with varying
164 moisture and sodium gradients was evaluated. The latter cheeses were produced by (1) varying the
165 salting method, (2) varying the holding time of the cheese in the brine, and/or (3) varying the
166 holding time of the cheese in storage at 4 °C, relative to the control procedure. Table 2 presents an
167 overview of the cheese treatments per experiment, and the number of cheese blocks sampled.

168

169 2.4.1. Varying the salting method

170 Mozzarella curd was removed from the industrial line after dry-salting to about 0.9 %, w/w, molded,
171 vacuum-packed, and stored at 4 °C. Cheeses that were produced following this protocol are further
172 referred to as dry-salted only (DS) cheese. One control and one DS cheese block were randomly
173 selected from the same cheese vat, and stored at 4 °C for 3 d until they were assayed for
174 composition and ^{1}H- and ^{23}Na-profilometry (Table 2).

175

176 2.4.2. Varying the brining time

177 Mozzarella curd was dry-salted to about 0.9 %, w/w, and molded into cheese blocks. After molding,
178 one cheese block was vacuum-packed and stored directly at 4 °C (no brining), whereas seven other
179 cheese blocks from the same cheese vat were submerged into brine of about 20 %, w/w NaCl at
180 4 °C. One cheese block was removed from the brine after 0.5, 1, 2, 3, 4, 8 or 24 h of brining, rinsed
181 with water, vacuum-packed, and stored at 4 °C. Each cheese block was assayed for composition
182 and ^{1}H- and ^{23}Na-profilometry after 3 d of storage time at 4 °C (Table 2).
183
184 2.4.3. Varying the storage time
185 Eight control mozzarella cheese blocks were sampled from one cheese vat, and stored at 4 °C.
186 During storage, one cheese block was randomly selected after 2, 4, 9, 16, 24, 31, 37 or 44 d, and
187 assayed for ^{1}H- and ^{23}Na-profilometry. Two cheese cylinders were sampled by gently pushing the
188 sharpened edge of the NMR tube once vertically into the central part of the cheese block (length
189 ~80 mm), and once horizontally (length ~100 mm), in order to examine the influence of the
190 sampling orientation on the ^{1}H and ^{23}Na signal intensity.
191 The composition of the cheese was assayed after 2, 4, 9 and 16 d as storage time had no influence
192 on the total moisture, fat, protein or salt content of the cheese (Table 2) (To et al., 2020).

3. Results and discussion

3.1. Method evaluation

3.1.1. Influence of Na concentration on the signal intensity

Figure 1 illustrates the ^{1}H and ^{23}Na signal intensity of 2.3, 4.7, 7.0, 9.3 and 11.6 %, w/w $CH_3COONa.3H_2O$ solutions as measured via ^{1}H- and ^{23}Na-NMR relaxometry. The sodium concentrations in these solutions corresponded to 1.0, 2.0, 3.0, 4.0 and 5.0 %, w/w NaCl solutions, respectively. Increasing the concentration of $CH_3COONa.3H_2O$ in the solution slightly decreased the ^{1}H signal intensity (Fig. 1A), whereas it increased the ^{23}Na signal intensity proportionately (Fig. 1B). The decrease in ^{1}H signal intensity likely reflects a decrease in proton density (Table 1). Based on the composition and the density of the prepared salt solutions, the proton density was calculated and was shown to decrease with about 0.12 mg ^{1}H mL^{-1} for each %, w/w increment of $CH_3COONa.3H_2O$ (Table 1). This expected decrease in proton density was fitted to the experimental data by minimizing the sum of squared differences between corresponding experimental and theoretical values, and yielded a theoretical fit with slope equal to -1.17 a.u. per %, w/w and intercept equal to 1085 a.u. (full black line, Fig. 1A). The experimental data was also fitted to a linear regression model which yielded a straight line with slope and intercept (± 95% confidence interval) corresponding to -20.7 ± 53.5 a.u. per %, w/w and 1221 ± 413 a.u., respectively. Hence, the linear model, drawn from the experimental data, was not significantly different from the theoretical fit which was based on the expected linear decrease in proton density.

3.1.2. Resolution

In order to determine the 1D resolution, we measured the profile of a tube which was sealed from one side and filled with 3 M NaCl solution. The results are given in Figures 2A-B. As can be seen, a linear increase is measured over a certain region due to the 1D resolution for both ^{1}H and ^{23}Na.

Based on these measurements, the 1D resolution for ^{1}H (~2.5 mm) and ^{23}Na (~7.9 mm) was determined. The difference in 1D resolution for ^{1}H and ^{23}Na is likely attributed to the gyromagnetic factor which yields different values for ^{1}H and ^{23}Na at the same magnetic gradient (0.3 T M^{-1}). These resolutions where chosen as a compromise between the optimal resolution wanted and the signal-to-noise ratio.

3.1.3. Repeatability of the measurement

Figure 3A illustrates the ^{1}H and ^{23}Na signal intensity profiles measured on control cheese sampled after 1 month of storage at 4 °C. An increase in ^{1}H signal intensity between the first two measurements and the subsequent 8 measurements was observed, which likely reflects variations in sample temperature. Measuring the same sample ten times in succession did not alter the ^{23}Na signal intensity profile. In order to reduce the variability owing to sample deformations and/or fluctuations in sample temperature, we normalized the ^{1}H profile by dividing each value by the maximum ^{1}H-intensity value, and took the ratio of the ^{23}Na signal intensity and the corresponding normalized ^{1}H signal intensity value. The result is a normalized ^{1}H signal profile and a sample deformation-corrected ^{23}Na signal profile (Fig. 3B).

3.2. Evaluating the ability of the method to distinguish between cheeses with different zonal variations in moisture or salt content

3.2.1. Varying the salting method

Differences in the moisture, salt and S/M content of the control and DS cheeses were found (Table 2). Relative to the control, the moisture content increased from about 46 to 48 %, w/w when cheese was only dry-salted, molded, and air-cooled at about 4 °C. In addition, the fat and protein content reduced slightly from 22.3 to 22.0, and from 26.3 to 25.8 %, w/w (Table 2). Guinee, Mulholland,

241 Mullins & Corcoran (2000) imparted salt in low-moisture mozzarella via (1) dry-salting the curd
242 to about 4.6 %, w/w NaCl before curd plasticization, (2) via submerging unsalted cheese blocks
243 into brine comprising 23 %, w/w NaCl for about 6.5 h, or (3) via dry-salting the curd to about 1.0 %,
244 w/w NaCl before curd plasticization, and holding the salted curd after molding into brine
245 comprising 23 %, w/w NaCl for about 2.0 h. Guinee et al. (2000) found no significant differences
246 in the moisture, fat or protein content among cheeses salted via the latter two methods, but found
247 that the first method increased cheese moisture by about 3%, w/w, and decreased both fat and
248 protein content by about 1.5%, w/w. The addition of 4.6 %, w/w dry-salt before curd plasticization
249 increased the S/M content of the cheese during curd plasticization, and likely promoted
250 solubilization of colloidal calcium via exchange with aqueous sodium, and hence, augmented
251 protein hydration (Guinee et al., 2000; Smith et al., 2017; Sood, Gaind & Dewan, 1979). The
252 interstudy discrepancy, i.e., the relatively small influence of adding dry-salt to the curd after
253 plasticization on the amount of fat and protein retained in the cheese as seen in the current study,
254 may be explained by differences in the salting procedure, i.e., the amount of dry-salt added to the
255 curd was lower (0.9 %, w/w), and dry-salt was added after curd plasticization (Guinee et al., 2000).
256 Figure 4A illustrates the normalized ^{1}H profile of the control (46.3 %, w/w moisture, 1.5 %, w/w
257 salt; full black line) and a DS mozzarella (48.3 %, w/w moisture, 0.8 %, w/w salt; bracketed line)
258 after 3 d of storage at 4 °C. As can be seen, the control mozzarella exhibited a normalized ^{1}H
259 profile with no clear ^{1}H gradient after 3 d of storage at 4 °C, indicating that the migration of
260 moisture throughout the cheese was limited. Figure 4A furthermore illustrates an increasing ^{1}H
261 signal intensity in DS mozzarella with 0.8 %, w/w salt when measuring from 45 to 130 mm, which
262 reflected the size of the sample which increased from bottom to top (Supplementary Table A.1),
263 and hence, indicates that the ^{1}H profiles can be used to correct the intensity values obtained during
264 ^{23}Na profiling for deviations from a perfectly cylindrical geometry.

265 The corrected ^{23}Na profiles are given in Figure 4B, and illustrate the variations in spatial salt
266 content between control (1.5 %, w/w NaCl) and DS mozzarella (0.8 % salt, w/w). As expected, the
267 latter cheese sample exhibited a uniform ^{23}Na distribution, whereas the control had the highest ^{23}Na
268 content at the surface and the lowest at the core. In addition, the ^{23}Na profiles of the control and
269 DS mozzarella overlapped in the region ranging from 75 to 115 mm, confirming that both cheeses
270 were dry-salted to a similar salt content during the manufacturing process (~ 0.9 %, w/w).
271 The larger ratio of the ^{23}Na signal at the surface of the control sample to that at its core (Fig. 4B)
272 to that presented earlier for cheese after 1 month of storage at 4 °C (Fig. 3B) reflects the influence
273 of storage time on the development of the salt gradient, which is discussed further in section 3.2.3.
274
275 3.2.2. Varying the brining time
276 Brining the cheese from 0 up to 24 h decreased the cheese moisture content from 48.3 to 45.4 %,
277 w/w, increased salt and S/M from 0.8 to 1.8 %, w/w, and from 1.6 to 3.9 %, w/w, respectively, and
278 slightly increased fat and protein content (Table 2). These findings suggest an inward diffusion of
279 sodium into the cheese and moisture moving outwards during brining in order to establish an
280 osmotic equilibrium (Fox et al., 2017, Luo, Pan, Guo, & Ren, 2013).
281 The measured ^{23}Na profiles given in Figure 5A represents the combined effects of brining time (0
282 to 24 h), and 3 d of storage time at 4 °C. Despite the influence of 3 d of storage time on the shape
283 of the profile relative to that measured directly after brining, a clear positive correlation between
284 the brining time and ^{23}Na signal intensity was found (Fig. 5A). The rate at which the ^{23}Na signal
285 intensity increased as a function of brining time was largest after about 0.5 h of brining, and slowed
286 down afterwards on increasing brining time to 24 h. This finding accorded with the proportional
287 increase in the salt content of the cheese with the square root of the brining time (Fig. 5B) (Guinee,

2004). The slower rate of salt uptake during prolonged brining was likely due to continuous dehydration of the cheese surface (Melilli et al., 2006).

No increase in ^{23}Na signal intensity was found at the core of the cheese after 24 h brining combined with 3 d of storage at 4 °C (Fig. 5A). The slow diffusion of ^{23}Na from the surface to the core was in agreement with other researchers who reported that the uptake of salt was impeded by cheese moisture with a high viscosity, surface free fat, occluded fat globules (tortuosity), protein hydration and consequent swelling, cross-diffusion of other solutes and the interactions thereof (Fox et al., 2017; Gerla & Rubiolo, 2003; Geurts, Walstra, & Mulder, 1974; Luo et al., 2013, Walstra et al., 1999).

3.2.2.1. Theoretical model to determine the effective diffusion coefficient

Mathematically, the diffusion of salt in the cheese matrix during brining could be described by Fick's 2nd law of diffusion using the semi-infinite cylinder approach with constant surface concentration by assuming a unidirectional non-steady mass transfer of cheese moisture and NaCl, and by setting the following boundary conditions (Floury, Jeanson, Aly, & Lortal, 2010; Walstra et al., 1999):

At $t = 0$ h ; $C = C_0$ for all $0 < x < \infty$

At $0 \text{ h} < t < 24$ h ; $C = C_b$ for $x = 0$; $C = C_0$ for $x = \infty$

With t = brining time; C = S/M in cheese at t; C_0 = S/M in cheese at t = 0 (i.e. before the start of the brining process); x = distance from the brine/cheese interface into the cheese matrix; C_b = S/M in brine. Based on Fick's 2nd law of diffusion the theoretical salt concentration profile at a given brining time t corresponds to Equation 1 (Crank, 1975):

$$C = C_0 + [1 - \text{erf}\,(x.(2\sqrt{(D^*.t)})^{-1})] \,.\, (C_b - C_0) \qquad \text{Eq. 1}$$

309 With D* representing the effective diffusion coefficient. The model based on Fick's 2nd law
310 predicts the highest salt content at the surface of the cheese which, depending on the brining time,
311 decreases more or less steeply as the distance from the surface of the cheese increases.
312 3.2.2.2. Experimental data
313 As the experimental ^{23}Na-profiles were obtained only 3 days after the brining process, Equation 1
314 cannot be directly fitted to our experimental data which clearly show a gradual increase in the
315 corrected ^{23}Na signal intensity close to the surface, before decreasing again (Fig. 5A). This
316 discrepancy is partly due to the effects of storage time, i.e., an inwards movement of the salt front
317 resulting from salt diffusion from the cheese surface to the core. Moreover, the limited resolution
318 of the ^{23}Na signal intensity measurement, which yields an average value of a cylindrical section of
319 ~7.90 mm length, and hence, smears out the high signal of a more narrow zone, further complicates
320 the direct comparison between the theoretical and experimental data. This smearing effect could
321 be circumvented by determining the total area below the corrected ^{23}Na signal intensity profile,
322 which provides an estimation of the total salt content, and is unaffected by the additional 3 days
323 equilibration period prior to the actual measurement.
324 Increasing the brining time indeed resulted in an increase in the area below the experimental curves,
325 and thus an increase in the total salt content of the cheese (Table 2, Fig. 5A-B). Besides, the
326 expected salt content (at a fixed brining time) for a specified effective diffusion coefficient could
327 be determined in a similar way by determining the area below the theoretical profile, as described
328 by Equation 1. Hence, for each brining time, the area below the theoretical concentration profile
329 was fitted to that below the experimental profile over a distance corresponding to that between the
330 surface of the cheese to its core while simultaneously resolving for D*.
331 3.2.2.3. Fitting the experimental data to the theoretical model

Figure 5C illustrates the theoretical fitted model as derived from Fick's 2nd law using a D* equal to 3.23• E^{-10} m^2 s^{-1} as a function of the square root of the brining time (full black line), and compares them with the experimental model (dashed line; $R^2 \sim 0.92$) derived from the experimental data (black data points). The slope and intercept (± 95% confidence interval) of the experimental model corresponded to 156 965 ± 53 750 (a.u.) m per $h^{(1/2)}$ and 494 244 ± 132 441(a.u.) m, respectively, and were not significantly different from those of the theoretical model [slope = 160 661 (a.u.) m per $h^{(1/2)}$; intercept = 485 140 (a.u.) m] (Crank, 1975; Walstra et al., 1999), suggesting that the applied approach was suitable for determining the D*.

Moreover, the derived D* was consistent with those reported by Luo et al. (2013), who examined the effective diffusion of Na^+ into high-moisture mozzarella during brining, and found D* values ranging between 6.19• E^{-12} and 1.98• E^{-9} m^2 s^{-1} depending on the duration of brining (0 to 24 h) and distance from the surface of the mozzarella cheese. Others have reported D* values for NaCl during brining of various semi-hard cheeses ranging from 1.5• E^{-10} to 4.5• E^{-10} m^2 s^{-1} (Bona, da Silva, Borsato, Silva, & Fidelis, 2007; Floury et al., 2010; Fox et al., 2017; Walstra et al., 1999). The large range of values reflects the diversity in the uptake of salt in cheese during brining, which is undoubtedly related to differences in cheese processing, brining conditions, composition, structure, volume or in modeling approach (Floury et al., 2010; Giroux, Lemaire & Britten, 2022). The approach used in this study, i.e., an adapted version of Fick's 2nd law of diffusion, was chosen for its simplicity and because the objective was to verify our experimental D* against those reported for other semi-hard cheeses. By comparison, the diffusion coefficient of NaCl in water is an order of magnitude larger: it ranges from $12•10^{-10}$ to $15•10^{-10}$ m^2 s^{-1} (Vitagliano & Lyons, 1955; Walstra et al., 1999).

3.2.3. Varying the storage time at 4 °C

Figure 6A illustrates a clear increase in the ^{23}Na signal intensity at the core of the control cheese on prolonging the storage time from 2 to 44 d, and suggests that salt distribution became more uniform during storage, likely because of the osmotic pressure gradient between the surface and the core of the sample (Everett, Rowney, Hickey & Roupas, 2004; Fox et al., 2017). However, an unexpected increase in surface area below the corrected ^{23}Na intensity profiles during storage from 0 to 44 d was found (Fig. 6B). Although we did not examine the variability in total NaCl between the cheeses measured on different days, previous research (To et al., 2022) has shown that the average coefficient of variation in total NaCl was about 10 % when cheeses were sampled from the same vat at the same mozzarella plant. Hence, the slight variability in total sodium between the different cheeses could not explain the more than twofold increase in surface area below the corrected ^{23}Na intensity profiles during storage.

The sodium signal intensity is determined by the spin density (ρ), time-to-echo (TE), repetition time (TR), longitudinal relaxation time (T_1), and transverse relaxation time (T_2) (Eq. 2).

$$I_{Na} = \rho \cdot (e^{-TE/T2}) \cdot (1-e^{(-TR/T1)}) \qquad \text{Eq. 2}$$

In the current study, the 2nd term in Equation 2 can be neglected as the repetition time was sufficiently large (50 ms), and hence, an increase in the signal intensity may be explained by an upwards shift in the T_2 relaxation time of ^{23}Na, which would indicate that the mobility of the ^{23}Na ion increased during storage. The distinction between two pools of ^{23}Na ions with different T_2 relaxation times has been reported by Gobet et al. (2010) who found fast (~ 3 ms) and slow T_2 (~ 11 ms) relaxation times for ^{23}Na in cheese. An increase in ^{23}Na ion mobility during storage may reflect storage-related changes in cheese biochemical properties, i.e., increases in protein hydration, solubilization of colloidal Ca-P, and hydrolysis of the protein (Smith et al., 2017; To et al., 2020).

3.2.3.1. Theoretical model to determine the effective diffusion coefficient

Assuming that the shift in T_2 relaxation time of ^{23}Na during storage was similar throughout the whole sample, we could estimate the D* of ^{23}Na from the changes in the salt gradient during storage, i.e., fitting the experimental data to a mathematical expression that describes the diffusion of NaCl initially confined in a plane source region of length 'h' with unidirectional non-steady mass transfer of NaCl, with the following boundary conditions (Crank, 1975):

At $t = 0$; $C = C_0$ for $x < h$; $C = 0$ for $x > h$

At $t > 0$; $C = C_{t, x < h}$; $C = C_{t, x > h}$

With t = storage time; C = S/M content; C_0 = S/M at the cheese surface exposed to brine at t = 0; x = distance from the cheese surface to the direction of the core.

The theoretical salt concentration-distance profile after various storage times is then described by Equation 3 with D* representing the effective diffusion coefficient (Crank, 1975).

$$C / C_0 = ½ [\text{erf} ((1-(x/h))/ \sqrt{(4D^*t/h^2)}) + \text{erf} ((1+(x/h))/ \sqrt{(4D^*t/h^2)})] \quad \text{Eq. 3}$$

This model describes how the salt content at the surface of the cheese ($x \leq h$) equals C_0 at t = 0 and decreases gradually for $t > 0$, whereas the salt content at $x > h$ equals 0 at t = 0 and increases gradually for $t > 0$.

From Figure 6A, we estimated h as the average width of the ^{23}Na signal intensity peak after 2 d of storage (h ~ 12 mm), and the distance between the surface and the core of the cheese as ~ 40 mm. C/C_0 as a function of x/h was then plotted for t = 2, 4, 9, 16, 24, 31, 37 or 44 d. For each storage time, the ratio of C/C_0 at x = 0 mm (surface of the sample) and at 40 mm (core of the sample) represents $C_{Na,surface}/C_{Na,core}$. The D* of ^{23}Na was then resolved by fitting the theoretical ($C_{Na,surface}/C_{Na,core}$) to the experimental data during storage.

3.2.3.2. Experimental data

399 The experimental data ($I_{Corrected\ Na,\ surface}/I_{Corrected\ Na,core}$) derived from Figure 6A represents the
400 changes in the salt gradient during storage of cheese that was manufactured by first dry-salting to
401 a target level of 0.9 %, w/w NaCl, followed by holding the dry-salted molded curd into brine
402 comprising 20 %, w/w NaCl for about 6 to 8 h.
403 The amount of dry-salt present in this cheese resulted in a positive ^{23}Na signal intensity value at
404 the core of the cheese (Fig. 6A), and hence, the data illustrated in Figure 6A was first zeroed by
405 subtracting the average ^{23}Na signal intensity ($5.41 \bullet E^{6}$ a.u) of DS cheese, i.e., cheese that was dry-
406 salted to the same target level of 0.9 %, w/w NaCl (Figure 5A). After zeroing, the values of the
407 ^{23}Na signal intensity values were normalized (Fig. 6C), and the ratio of $I_{Corrected\ Na,\ surface}/I_{Corrected}$
408 $_{Na,core}$ after each storage time was derived as the average of the two peak signal intensities of one
409 half (40 to 90 mm) of the cheese block and of the second half (90 to 150 mm), to the average of
410 the signal intensities in the cheese measured at the core from 80 to 100 mm.
411 3.2.3.3. Fitting the experimental data to the theoretical model
412 Figure 6D illustrates the experimental $I_{Corrected\ Na,\ surface}/I_{Corrected\ Na,core}$ as a function of storage time
413 to which the theoretical model was fitted using a D* equal to $5.21 \bullet E^{-10}$ m^2 s^{-1}. This value was
414 approximately 1.6 times larger than the one determined from the brining experiment ($3.23 \bullet E^{-10}$ m^2
415 s^{-1}) but was of a similar order of magnitude. The slight difference may be caused by differences in
416 the shift in the mobility of ^{23}Na at the core and surface of the sample during storage at 4 °C.
417 Figure 6D furthermore illustrates how the salt gradient decreased rapidly in the first 9 d after
418 manufacture, approached unity (bracketed line) after 24 - 31 d of storage at 4 °C and did not
419 significantly change afterwards, suggesting that the time required for the salt to more or less
420 distribute evenly throughout the cheese was approximately 1 month of storage at 4 °C, which was
421 similar to those reported for other firm/semi-hard cheeses, such as Edam cheese (2.5 kg, ~46%
422 moisture) (Guinee & Fox, 2017; Walstra et al., 1999).

3.2.3.4. Influence of sampling orientation

Figure 7 indicates that the ^{23}Na profile was not affected by sampling the cheese vertically or horizontally after 4 or 44 d of storage at 4 °C. The difference in profile length reflects the difference in the height of the sampled cheese cylinders, i.e, about 80 or 100 mm, respectively. (Supplementary Table A.1), which follows from the dimensions of the cheese blocks. This findings suggests that the direction of the protein fibers did not influence the intensity of the ^{23}Na signal and the salt gradient when measuring LMPS mozzarella cheese that was brined for 6 to 8 h at 4 °C.

4. Conclusions

We investigated the use of ^{1}H and ^{23}Na NMR relaxometry, combined with a stepper motor to load the sample, as an alternative tool for conventional salt quantification methods, and to evaluate zonal variations in moisture or salt content in commercial LMPS mozzarella cheese.

The ^{23}Na signal intensity was found to increase with the level of salt in the cheese which enabled to visualize the salt gradient in cheese, and hence, to distinguish dry-salted from brined cheese in a fast and non-invasive way: about 21 min was required to measure the ^{1}H- and ^{23}Na- profile of the cheese over a distance of about 10 cm using the applied conditions.

The experiments on cheeses brined for different times revealed that the effective diffusion coefficient of NaCl was about $3.23 \bullet 10^{-10}$ m^2 s^{-1} during LMPS mozzarella brining, which was of the same order of magnitude as the values reported for various other semi-hard cheeses, suggesting that the experimental results corresponded favorably with Fick's 2nd law of diffusion.

The applied methodology furthermore indicated that the salt gradient of brined LMPS mozzarella was more or less equilibrated after 1 month of storage at 4 °C, and that the salt gradient decreased most rapidly in the first week post-manufacture, and more slowly afterwards. From the obtained data during 44 d of storage at 4 °C, an effective diffusion coefficient of NaCl of about $5.21 \bullet 10^{-10}$ m^2 s^{-1} was derived.

The zonal variations in ^{1}H signal intensity were mainly due to non-uniform sample thickness, whereby small variations in moisture content in the cheese could not be distinguished from the random fluctuations in ^{1}H signal intensity, suggesting that protons associated with fat or protein protons may also have contributed to the signal intensity. Further research involving the optimization of the applied method, i.e., improving the resolution of the ^{23}Na-signal intensity, or the influence of storage time on the zonal T_2 relaxation time of ^{23}Na ions, is recommended to enable rapid and accurate assessment of the salt gradient in mozzarella and other brined cheeses.

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Declaration of competing interest

None.

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595 **LIST OF CAPTIONS**

596 **Fig. 1** Signal intensity profiles of aqueous sodium acetate solutions as measured by (A) ^{1}H-NMR
597 and (B) ^{23}Na-NMR. Black dots represent the experimental data. The full black line represents the
598 theoretical decrease in proton density with increasing concentration of sodium acetate fitted to the
599 experimental data. The dashed line represents the model fitted to the experimental data using linear
600 regression analysis.

601

602 **Fig. 2** (A) ^{1}H and (B) ^{23}Na signal intensity profiles of a PET tube which was sealed from one side
603 and filled with 3 M NaCl solution. The dashed horizontal lines represent the minimum (bottom
604 baseline) and maximum signal intensity (top baseline). The bracketed lines represent the tangent
605 drawn through the point of maximum slope. The dashed vertical lines represent the sample distance
606 contributing to the signal intensity measurement (resolution).

607

608 **Fig. 3** (A) ^{1}H and ^{23}Na signal intensity profile of control cheese stored for 1 month at 4 °C. The
609 sample was profiled 10 times in succession to examine the repeatability of the measurement. (B)
610 Normalized ^{1}H and corrected ^{23}Na signal intensity profiles of the same control cheese.

611

612 **Fig. 4** Signal intensity profiles of control (1.5 %, w/w NaCl; full black line) and dry-salted (DS;
613 0.8 w/w NaCl; bracketed line) cheese measured by (A) ^{1}H-NMR and subsequent normalization of
614 the obtained values and (B) ^{23}Na-NMR profilometry and subsequent correction of the obtained
615 values after 3 d of storage at 4 °C.

616

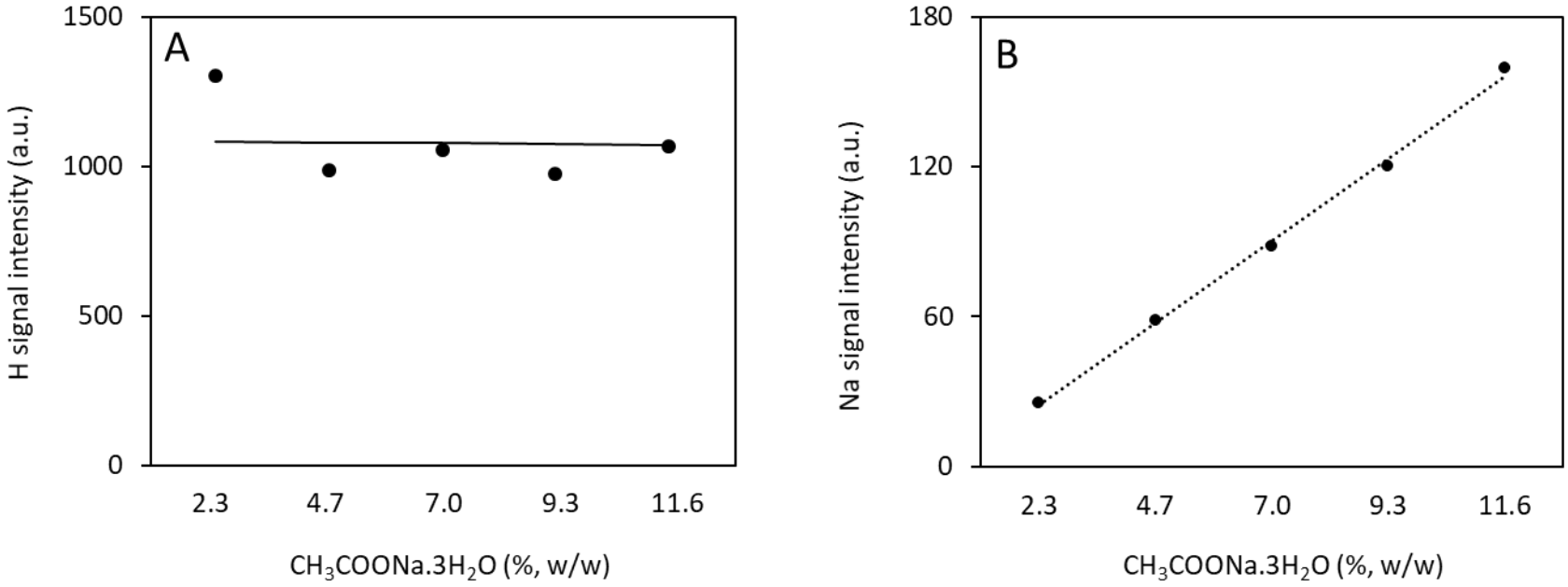
617 **Fig. 5** (A) Influence of increasing brining time from 0 to 24 h on the corrected ^{23}Na signal intensity
618 of control cheese after 3 d of storage at 4 °C. (B) Linear relationship between the average salt

content in the cheese and the square root of the brining time. The dashed line presents the model fitted to the experimental data using linear regression analysis. (C) Area below the ^{23}Na intensity profile from the surface to the core of commercial low-moisture part-skim mozzarella stored for 3 d at 4 °C as a function of the square root of the brining time. Black dots represent the experimental data, whereas the dashed line represents the model fitted to the experimental data using linear regression analysis. The experimental model was compared with the theoretical model (full black line) based on Fick's 2nd law of diffusion after curve fitting, and using an effective diffusion coefficient (D*) equal to 3.23 10^{-10} m^2/s.

Fig. 6 (A) Influence of storage time at 4 °C on the corrected ^{23}Na signal intensity of control cheese after 6 to 8 h of brining at 4 °C. (B) Increase in the total surface area below the corrected ^{23}Na signal intensity profile as a function of storage time at 4 °C. (C) Influence of storage time at 4 °C on the corrected ^{23}Na signal intensity of control cheese after 6 to 8 h of brining at 4 °C after zeroing and normalizing the data. (D) Decrease in the ratio of the corrected ^{23}Na signal intensity at the surface to that of the core as a function of storage time. The experimental data (●) were compared with a theoretical model (○) after curve fitting using an effective diffusion coefficient (D*) equal to 5.21 10^{-10} m^2/s. The bracketed line corresponds to a ratio equal to 1 which reflects a uniform salt gradient.

Fig. 7 Corrected ^{23}Na signal intensity profile of control cheese, sampled vertically or horizontally, after 4 or 44 d of storage at 4 °C.

640 To Fig. 1



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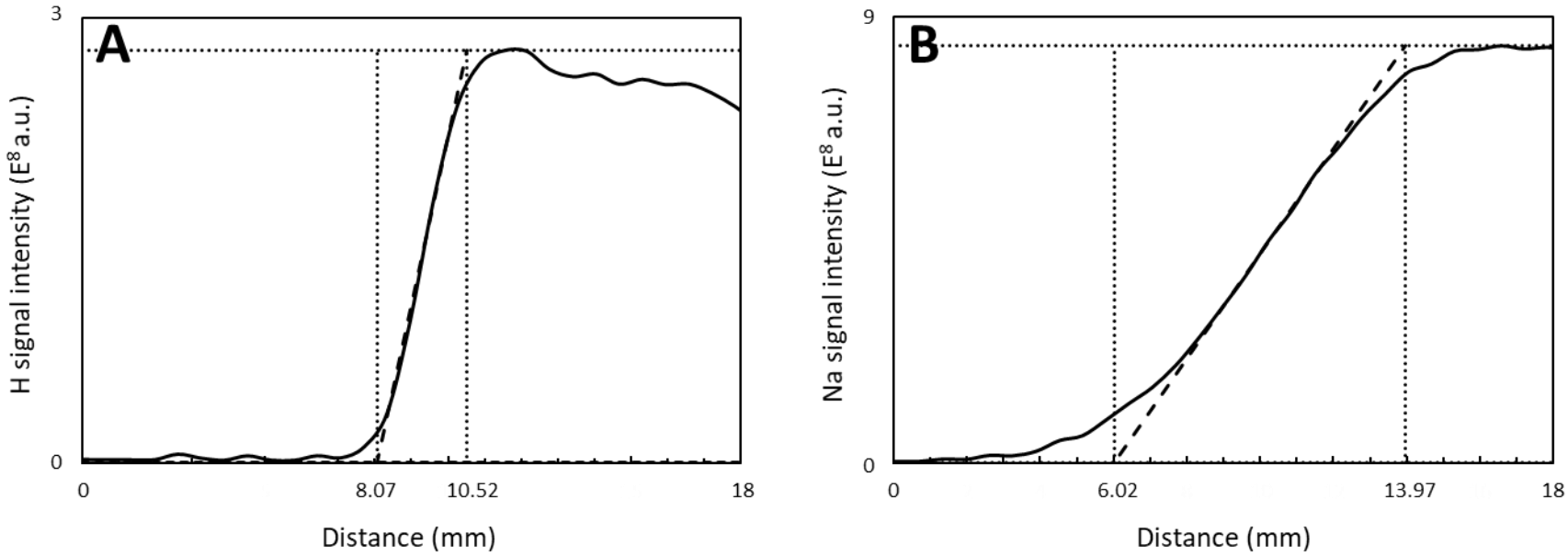
642 To Fig. 2

643



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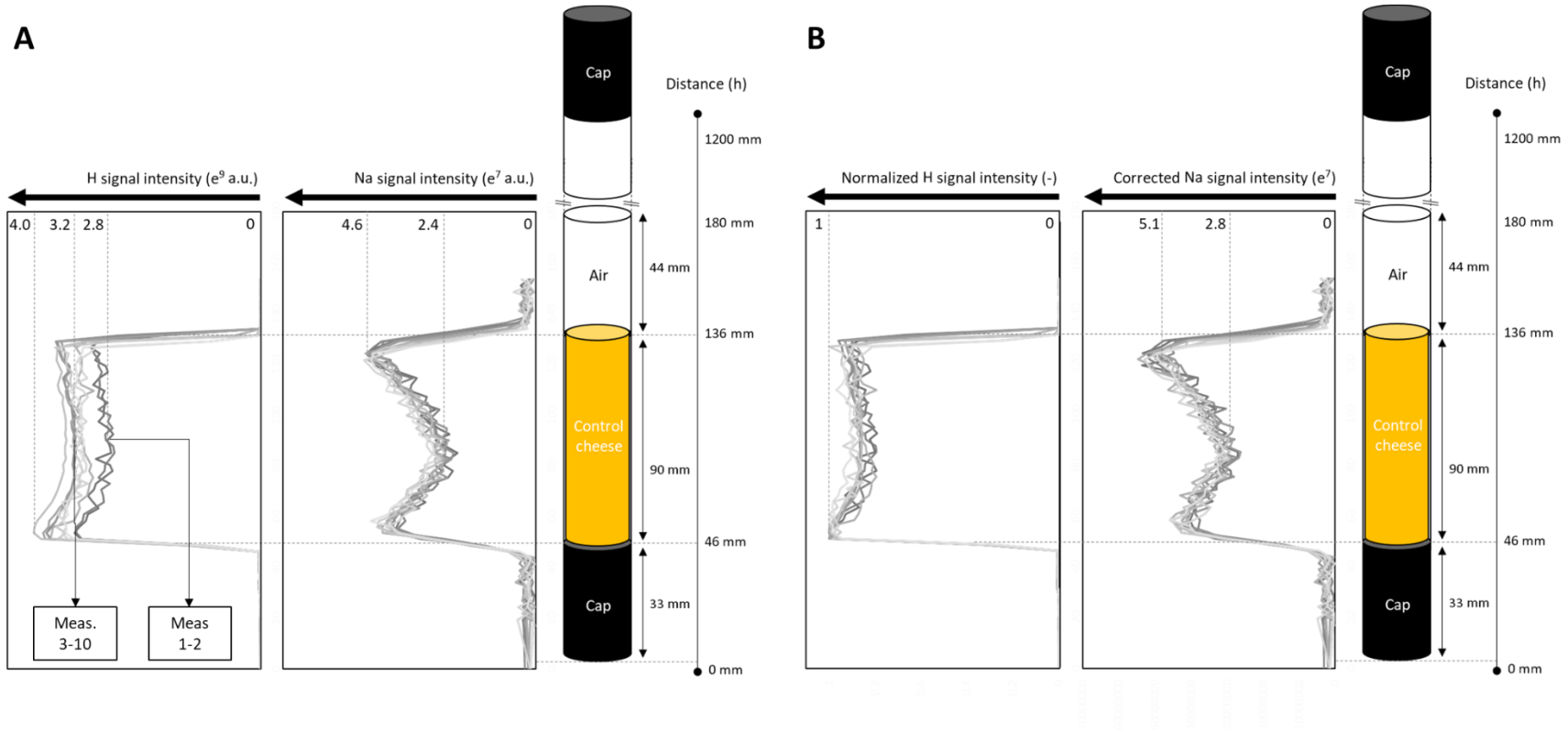
645 To Fig. 3



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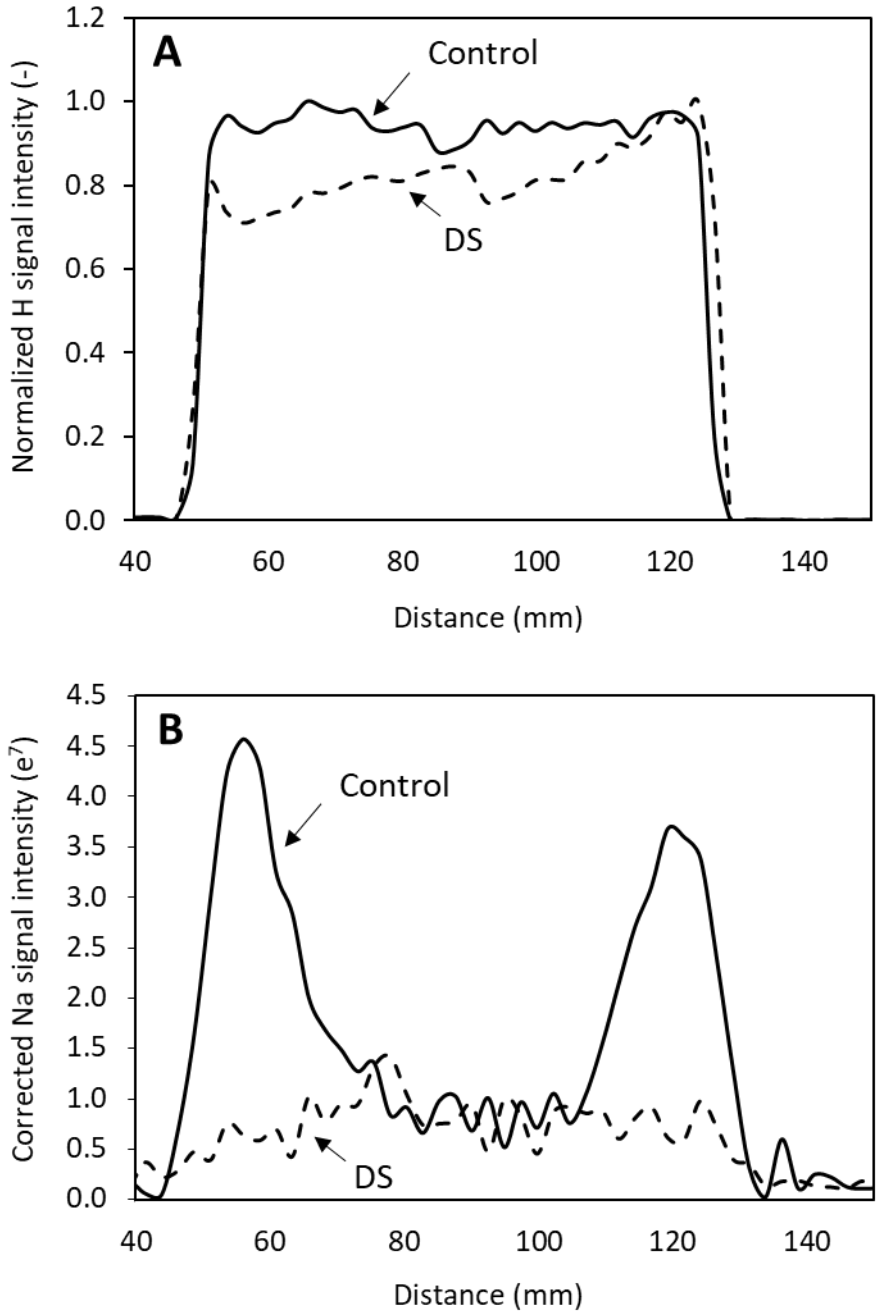
647 To Fig. 4



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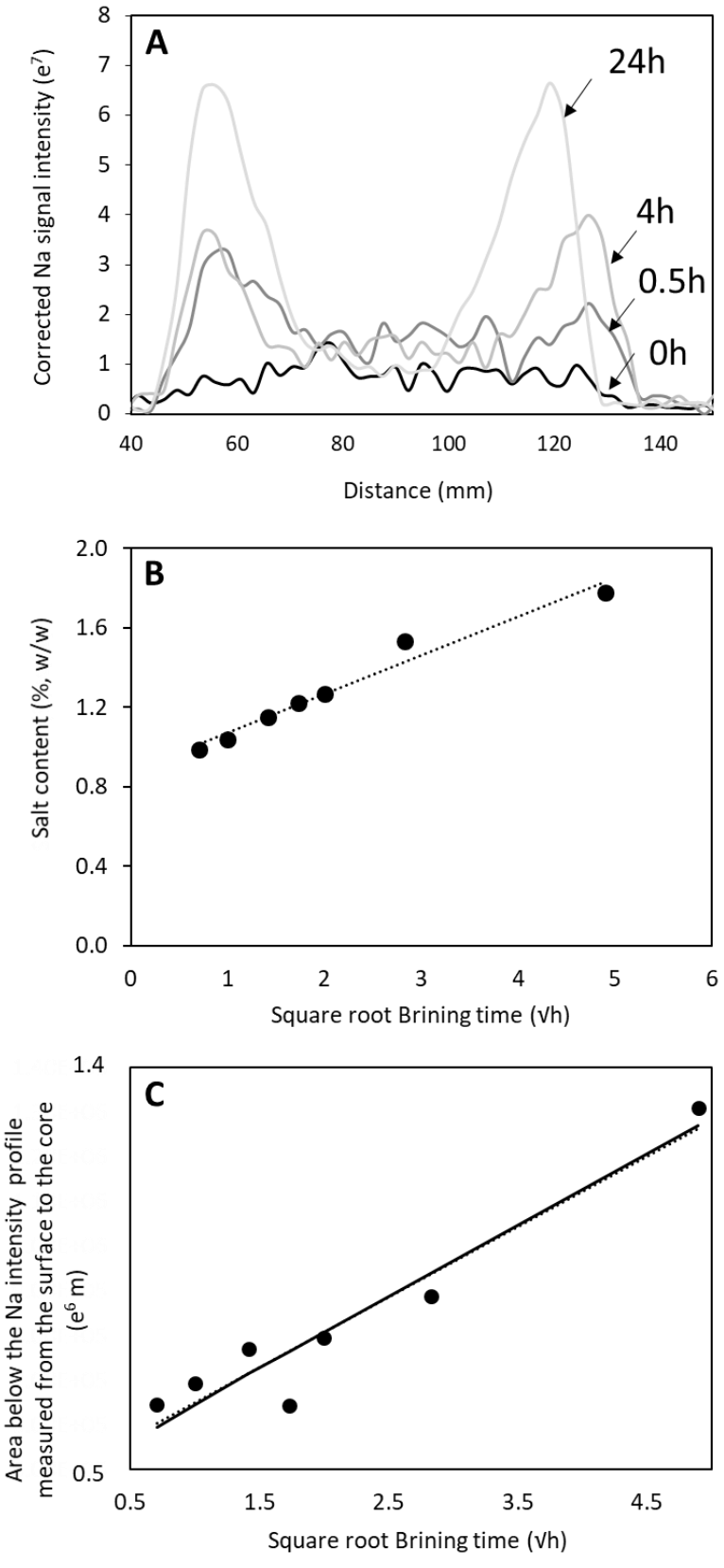

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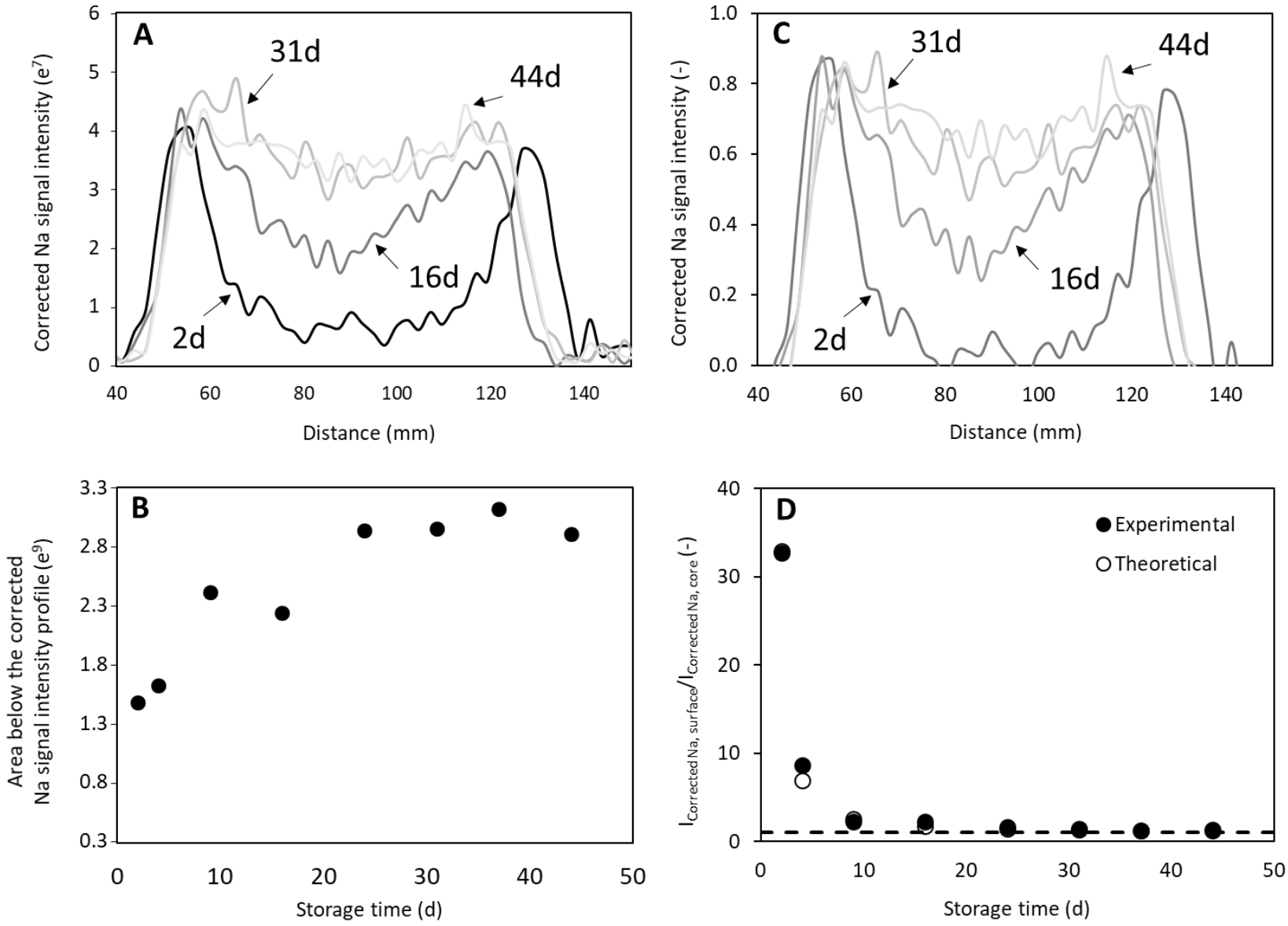
651 To Fig. 6



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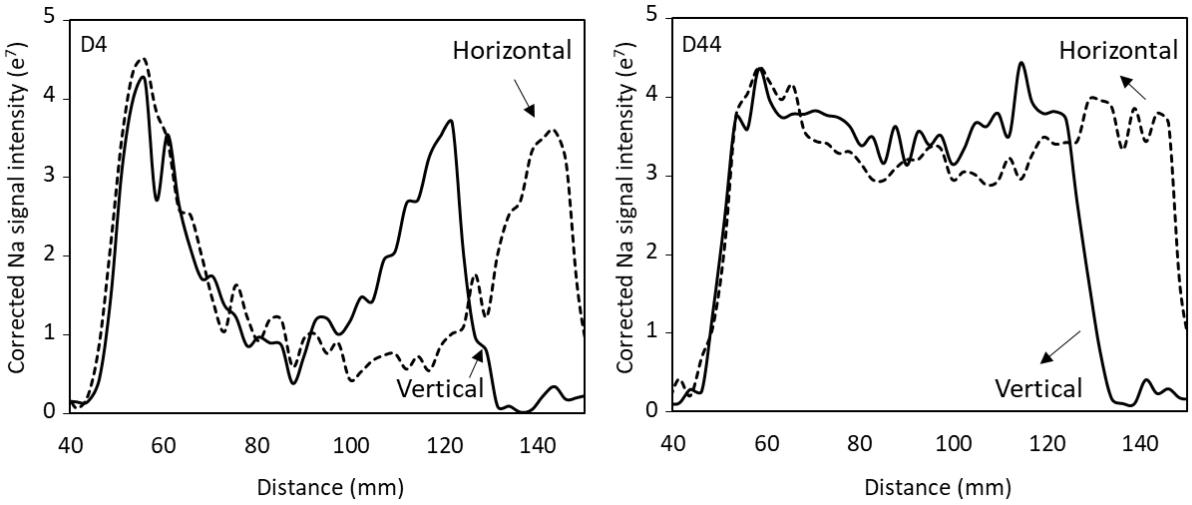
653 To Fig. 7



654

655 **Table 1.** Composition, density and proton density of $CH_3COONa.3H_2O$ salt solutions.

$CH_3COONa.3H_2O$ (%, w/w)	Density (g mL^{-1})	Proton density (g H mL^{-1})
2.3	1.005	0.11067
4.7	1.013	0.11041
7.0	1.022	0.11035
9.3	1.027	0.10985
11.6	1.035	0.10955

656

Table 2. Overview of the cheese treatments per experiment, the number of cheese blocks sampled, and the composition of the cheese.[ab]

Experiment	Cheese treatments			Number of cheese blocks	Cheese composition				
	Salting method	Brining time (h)	Storage time (d)		Moisture (%, w/w)	Fat (%, w/w)	Protein (%, w/w)	Salt (%, w/w)	S/M (%, w/w)
Salting method	DS + Br	6-8	3	1	46.3	22.3	26.3	1.5	3.3
	DS	0	3	1	48.3	22.0	25.8	0.8	1.6
Brining time	DS	0	3	1	48.3	22.0	25.8	0.8	1.6
	DS + Br	0.5	3	1	47.4	22.1	26.0	1.0	2.1
	DS + Br	1	3	1	47.4	22.0	26.1	1.0	2.2
	DS + Br	2	3	1	47.2	22.1	26.2	1.2	2.4
	DS + Br	3	3	1	47.2	22.2	26.2	1.2	2.6
	DS + Br	4	3	1	46.7	22.3	26.3	1.3	2.7
	DS + Br	8	3	1	46.3	22.3	26.3	1.5	3.3
	DS + Br	24	3	1	45.4	22.7	26.4	1.8	3.9
Storage time	DS + Br	6-8	2	1	47.0	22.4	26.0	1.2	2.6
	DS + Br	6-8	4	1	47.1	22.2	25.8	1.3	2.8
	DS + Br	6-8	9	1	46.6	22.4	26.2	1.2	2.6
	DS + Br	6-8	16	1	46.7	22.4	26.0	1.3	2.8
	DS + Br	6-8	24	1	-	-	-	-	-
	DS + Br	6-8	31	1	-	-	-	-	-
	DS + Br	6-8	37	1	-	-	-	-	-
	DS + Br	6-8	44	1	-	-	-	-	-

[a]Abbreviations are: DS, dry-salted; Br, brining; S/M, salt-in-moisture content.

[b]Low-moisture part-skim mozzarella was salted by dry-salting the curd to a target value of 0.9 %, w/w NaCl and molding the curd into cheese blocks. The molded blocks were held in brine comprising 20 %, w/w NaCl at 4 °C for different periods (brining time). After brining, the cheese blocks were vacuum-packed and held at 4 °C for different periods (storage time) prior to analysis.

Supplementary Table A.1. Dimensions of the sampled cheese cylinders.[ab]

Experiment	Cheese treatments			Number of cheese blocks	Dimensions of the sampled cheese cylinder			
	Salting method	Brining time (h)	Storage time (d)		$\phi_{bottom,\ sample}$ (mm)	$\phi_{center,\ sample}$ (mm)	$\phi_{top,\ sample}$ (mm)	Sample length (mm)
Salting method	DS + Br	6-8	3	1	23.9	23.7	24.1	81.6
	DS	0	3	1	23.1	24.1	24.7	83.7
Brining time	DS	0	3	1	23.1	24.1	24.7	83.7
	DS + Br	0.5	3	1	24.6	22.6	24.6	86.4
	DS + Br	1	3	1	24.6	23.1	24.8	86.4
	DS + Br	2	3	1	24.4	24.1	24.4	80.5
	DS + Br	3	3	1	24.2	24.5	24.6	82.2
	DS + Br	4	3	1	24.5	24.7	24.8	86.0
	DS + Br	8	3	1	23.9	23.7	24.1	81.6
	DS + Br	24	3	1	24.8	25.0	24.9	80.3
Storage time	DS + Br	6-8	4	1	24.0	23.9	24.0	80.9
	DS + Br	6-8	4	1	24.4	24.1	24.3	102.2
	DS + Br	6-8	44	1	24.4	24.6	24.8	80.5
	DS + Br	6-8	44	1	23.8	23.8	24.4	102.6

[a]Abbreviations are: DS, dry-salted; Br, brining.

[b]Low-moisture part-skim mozzarella was salted by dry-salting the curd to a target value of 0.9 %, w/w NaCl and molding the curd into cheese blocks. The molded blocks were held in brine comprising 20 %, w/w NaCl at 4 °C for different periods (brining time). After brining, the cheese blocks were vacuum-packed and held at 4 °C for different periods (storage time) prior to assaying cheese cylinders for ^{1}H- and ^{23}Na-NMR profile analysis.