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Short communication

# Moisture sorption by dairy powders studied by low-field NMR

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ABSTRACT

The influence of moisture sorption on spin−spin ($T_2$) relaxation times for skim milk powder (SMP), milk protein concentrates (MPC50 and MPC80) and amorphous lactose was studied by low-field nuclear magnetic resonance (NMR). For amorphous lactose and MPC80, only one main peak was observed in the $T_2$ distribution, whereas for SMP and MCP50, two main peaks, relating to the water associated with the amorphous lactose and protein, were observed at $a_w$ <0.4; at $a_w$ >0.4 lactose crystallised and no peak relating to water associated with lactose was visible in the $T_2$ spectrum (water of crystallisation for crystalline lactose has shorter relaxation times, which cannot be detected by low-field NMR). $T_2$ peak position and peak area for the protein fraction increased with increasing $a_w$. Shifts in the $T_2$ peak position showed the transition from mono- and bilayer water at low $a_w$ to more rapidly exchanging forms of water at higher $a_w$.


## 1. Introduction

Removal of water is one of the most commonly applied processes to increase the shelf-life of milk and dairy ingredients, resulting in powdered dairy products such as skim milk powder (SMP), whole milk powders (WMP), milk protein concentrate (MPC) and whey protein concentrate (WPC). During the drying of these powders, typically by spray-drying, water content is reduced to values <5% (w/w), leading to water activity ($a_w$) values <0.3. Water activity is a crucial determinant of powder stability, as it governs the glass transition temperature ($T_g$), which relates to, e.g., development of stickiness and crystallisation of lactose (Huppertz & Gazi, 2016; Paterson Brooks, Bronlund, & Foster, 2005; Roos, 2002). Furthermore, development of insolubility, lipid oxidation and browning during storage also depend strongly on the $a_w$ of the powders (Fan et al., 2018). Therefore, water (de)sorption and water binding by dairy products is of great scientific and industrial relevance.

The relationship between water content and water activity, and therewith $T_g$, of products is typically expressed in the form of moisture sorption isotherms. Moisture content (often expressed as g water per g of dry matter) increases with $a_w$ but the manner in which this occurs is dependent on the components of the product. In the $a_w$ region 0.0−0.3, most water is in the form of so-called monolayer water, which is tightly bound to ionic and polar groups of e.g., proteins. In addition, hydration water around exposed hydrophobic residues is also present in this $a_w$-region (Rupley & Careri, 1991). These 'types' of water are typically strongly bound in highly ordered structures (Oleinikova, Smolin, & Brovchenko, 2007). Rotation and translation of water molecules in this layer is impaired, which on the other hand impacts nuclear magnetic resonance (NMR) relaxation time (Kuntz & Kauzmann, 1974). With increasing $a_w$ >0.3, layers of hydrogen-bonded water with progressively decreasing ordered structures, i.e., multilayer water, start surrounding the aforementioned monolayer water; at even higher $a_w$ (e.g., >0.5) capillary water held in structural openings can also be present and is again less structured and shows less impairment of rotation and translation of water molecules (Oleinikova et al., 2007). These differences in water mobility can be monitored readily by low-field NMR spectroscopy.

Low-field NMR has been frequently used to assess the molecular mobility of water as well as water-biopolymer interactions in food systems (Kou, Molitor, & Schmidt, 1999). In high moisture food systems, $^1$H NMR spectrum is dominated by the bulk water signal, thus providing very little information about water dynamics. On the contrary, food systems containing small amounts of water are characterised with a bulk water signal that is greatly depressed or even absent or greatly reduced and a much slower proton exchange

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due to immobilisation of water (Kou, Dickinson, & Chinachoti, 2000). NMR relaxometry has been used previously to establish rehydration properties of dairy powders (Davenel, Schuck, Mariette, & Brule, 2002; Schuck et al., 2002) and to link conformational changes of MPC to water mobility in dry state (Haque, Bhandari, Gidley, Deeth, & Whittaker, 2011). The relaxometry is based on differences in transverse relaxation times ($T_2$) of water molecules governed by molecular motion and exchange with protons on protein molecules. Therefore, it is possible to identify different populations of water molecules from their rotational mobilities and assign them to different environments (Hills, Manning, & Godward, 1999).

NMR studies on dry dairy systems are very limited mainly due to difficulties in data interpretation associated with structural and compositional complexity of samples bearing highly heterogeneous proton environments. The aim of the current study thus was to engage the NMR instrumentation frequently encountered in commercial settings in the assessment of water mobility in several commercial dry dairy samples and link that to water sorption properties of these powders. Main advantage of low-field NMR is in lower acquisition cost to that of a high-resolution NMR. The water mobility was assessed in samples equilibrated at different water activities ranging from <0.1 to 0.85 at 25 °C.

## 2. Materials and methods

### 2.1. Sample preparation

Low-heat skim milk powder, MPC50 and MPC80 were produced as described by Gazi and Huppertz (2015). Amorphous lactose was prepared by dissolving α-lactose monohydrate (Sigma–Aldrich, St. Louis, MO, USA) in deionised water at 50 °C at a concentration of 25 g 100 $g^{-1}$ water. The solution was subsequently flash-frozen in liquid nitrogen, freeze-dried and stored over phosphorus pentoxide. The absence of crystalline material was checked by polarised light microscopy. For adjusting the water activity of samples, aliquots were stored over phosphorus pentoxide and saturated solutions of lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, magnesium nitrate, sodium chloride and potassium chloride. Samples were stored for 3 weeks at room temperature after which water activity was determined.

### 2.2. Determination of moisture sorption isotherms

Moisture sorption isotherms for SMP, MPC50 and MPC80 were determined at 25 °C in the $a_w$ range 0.10–0.90 at intervals of 0.05 $a_w$ units using a Vapor Sorption Analyzer (VTI-SA+; TA Instruments, New Castle, DE, USA). The water content of each sample was plotted as a function of water activity. The Guggenheim-Anderson-de Boer (GAB) relationship was established by fitting the data applying the following equation:

$$\frac{m}{m_o} = \frac{C \cdot k \cdot a_w}{(1 - k \cdot a_w) \cdot (1 - k \cdot a_w + C \cdot k \cdot a_w)} \quad (1)$$

where m presents the water content (kg of water $kg^{-1}$ of dry matter), $m_0$ is the monolayer value of water molecules; and C, k are the constants.

### 2.3. Low-field NMR

The NMR relaxation measurements were conducted using a 23.5 MHz (0.54 T) MQC23-benchtop NMR (Oxford Instruments, Abingdon, UK). The NMR system was equipped with a temperature control device (Air Jet van SP industries model:XR11851) allowing temperature regulation between 10 °C and 40 °C. The samples upon equilibration were introduced into the instrument by filling approximately 2 g of powders into LR-NMR glass tubes. The measurements were conducted at 25 °C. The transverse relaxation time constants ($T_2$) of the samples were assessed by the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The CPMG experiments were carried out using a relaxation delay of 10 s and 32 consecutive scans for noise reduction along with a sweep width of 0.1 MHz. A total of 4096 echoes was collected, with a 90–180° pulse gap (τ) value of 100 μs. The resultant decays were analysed by Inverse Laplace Transform in the RI Win-DXP software (V. 1.2.3. Oxford Instruments).

## 3. Results and discussion

Moisture sorption isotherms were determined for SMP, MPC50 and MPC80, whereas sorption isotherm data for amorphous lactose were extracted from Bronlund and Paterson (2004). Sorption isotherms are shown in Fig. 1 and the GAB parameters derived therefrom are shown in Table 1. Only for MPC80 could sorption isotherms be determined up to $a_w = 0.9$. For the other amorphous lactose, SMP and MPC50, the occurrence of lactose crystallisation took place at $a_w$ >0.35, >0.55 and >0.65, respectively.

The occurrence of lactose crystallisation during sorption isotherm measurements and the occurrence thereof at higher $a_w$ with increasing protein and decreasing lactose content is in line with previous studies, which were reviewed by Huppertz and Gazi (2016). However, it should be noted that crystallisation at lower aw,

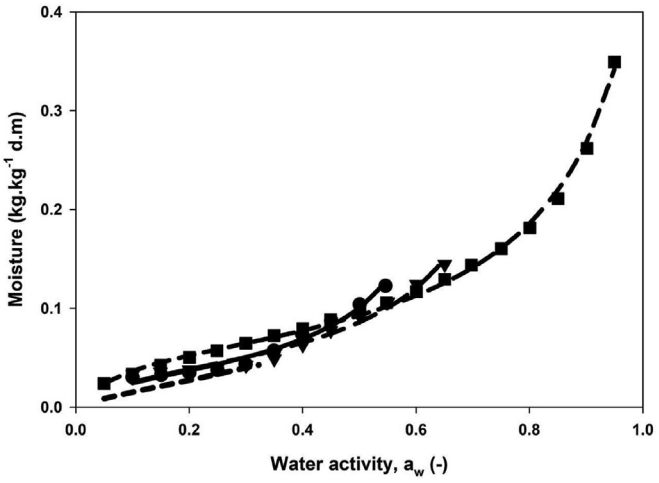


**Fig. 1.** Sorption isotherms of SMP (●), MPC50 (▼), MPC80 (■) samples. Amorphous lactose line (- - - -) was plotted based on the information derived from Bronlund and Paterson (2004).

**Table 1**
GAB isotherm parameters for SMP, MPC50 and MPC80.[a]

| Sample | $m_0$ | C | K | SSE |
|---|---|---|---|---|
| SMP | 0.0345 | 9.99 | 1.346 | 0.0002 |
| MPC50 | 0.0395 | 9.99 | 1.144 | 0.0003 |
| MPC80 | 0.0563 | 14.9 | 0.881 | 0.0003 |
| Amorphous lactose | 0.0488 | 3.23 | 1.16 | |

[a] Abbreviations are: $m_0$, monolayer value (kg of water $kg^{-1}$ of dry matter); C, k, constants; SSE, sum of squared estimate of errors. Parameters for amorphous lactose were extracted from Bronlund and Paterson (2004).

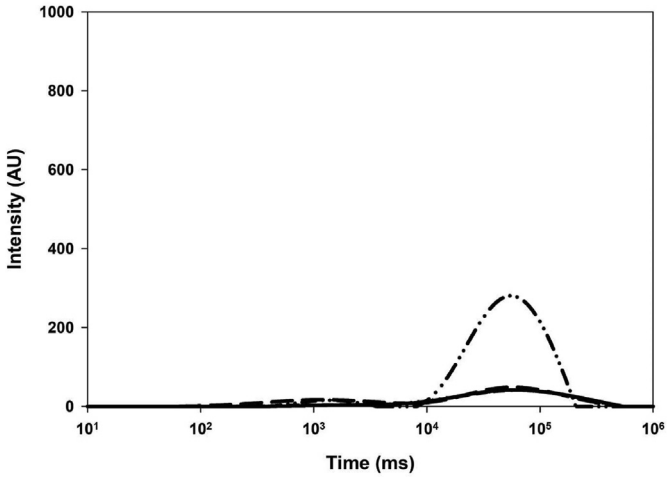


**Fig. 2.** $T_2$ distribution at different $a_w$ levels for (A) amorphous lactose: ——, <0.1; ·····, 0.144; ------, 0.217; — · · -, 0.323; - - - -, 0.432.

but above 0.35, can still occur on longer timescales because of delayed lactose crystallisation due to the presence of proteins and salts (Jouppila & Roos, 1994; Omar & Roos, 2007). Lactose crystallisation in dairy powders can occur due to increases in $a_w$ when $T > T_g$ with the rate of crystallisation often described as proportional to $T - T_g$ (Paterson et al., 2005). As $T_g$ increases with decreasing lactose content (Kelly et al., 2015), the aforementioned delay in lactose crystallisation for samples with lower lactose content is in line with expectations.

The $T_2$ spectra for the different samples at different $a_w$ values are shown in Figs. 2 and 3. For amorphous lactose (Fig. 2), the main peak was observed in the $T_2$-range $10^4$–$10^6$ ms, which strongly increased at $a_w = 0.32$, but decreased again at $a_w = 0.43$. The increase in peak intensity at $a_w = 0.32$ (Fig. 2) is due to moisture sorption by amorphous lactose and in line with the moisture sorption isotherm (Fig. 1). Although moisture sorption also occurs at lower $a_w$ (Fig. 1), at this point the adsorbed water is in the field of monolayer water, which has very short relaxation times, which are not detected by low-field NMR. The additional water absorbed at

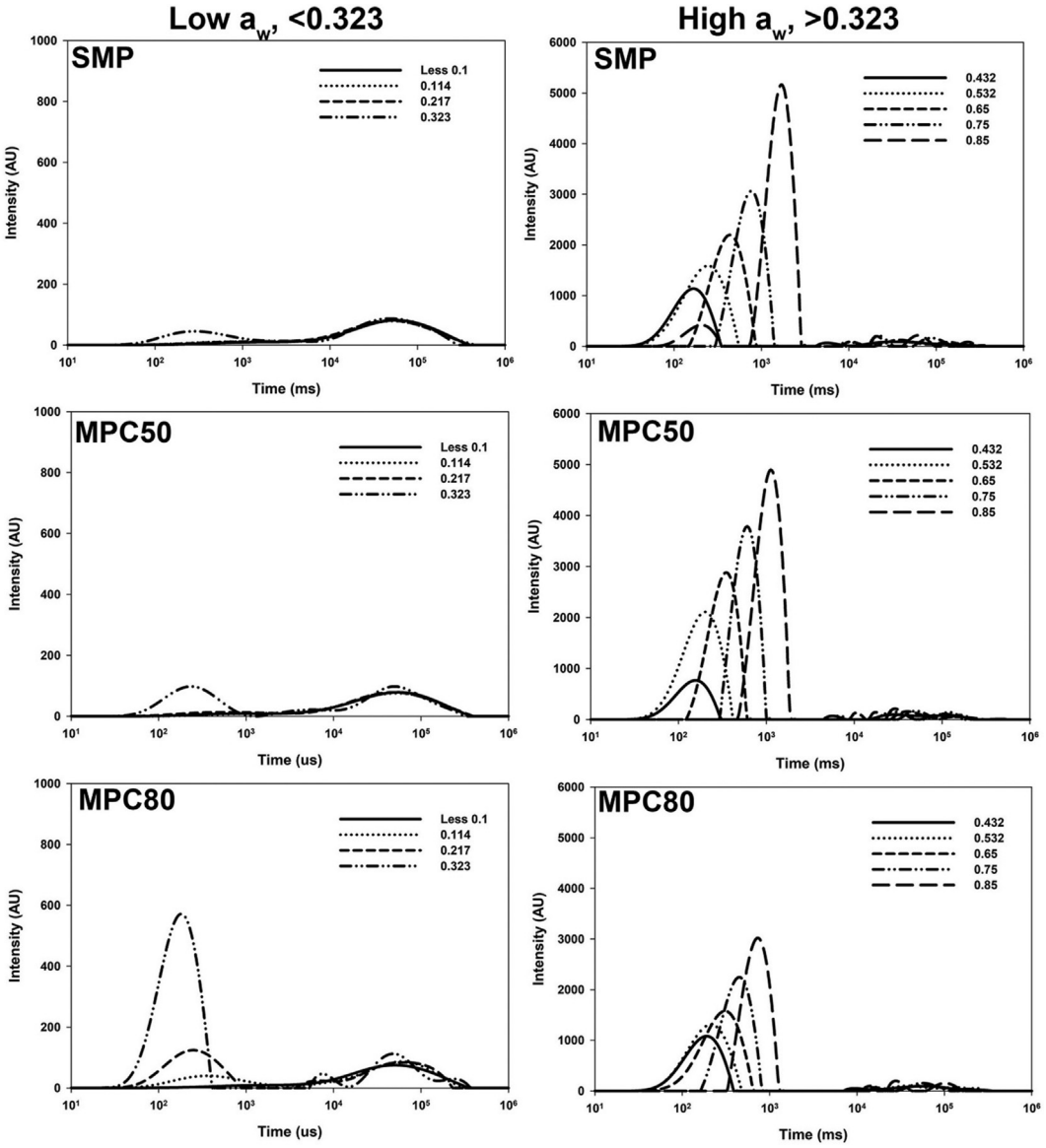


**Fig. 3.** $T_2$ distribution at different $a_w$ levels for SMP, MPC50, and MPC80.

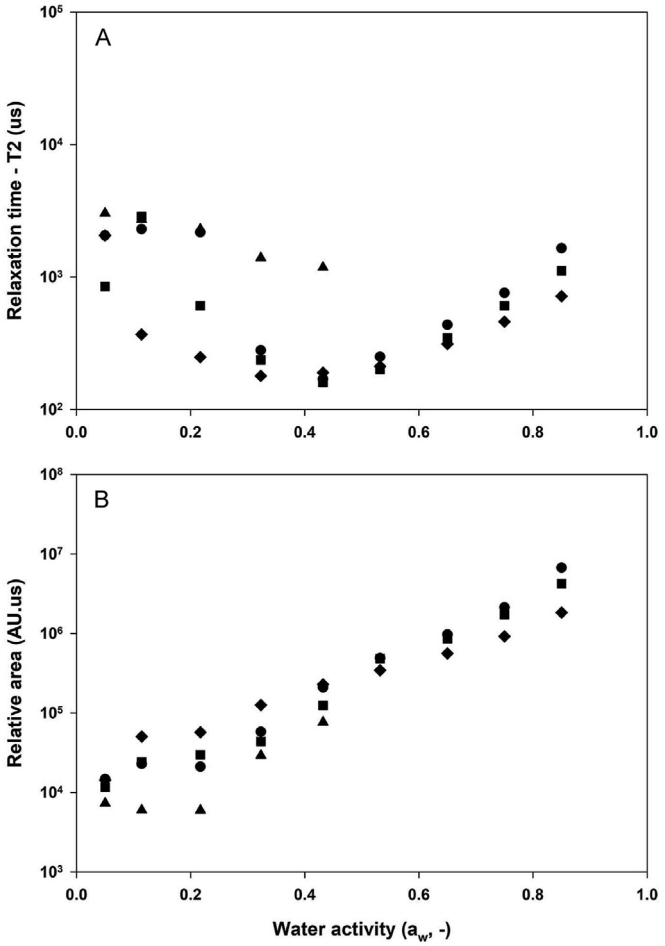


**Fig. 4.** (A) $T_2$ peak position, and (B) $T_2$ peak area of the protein peak as a function of $a_w$ for SMP (●), MPC50 (■), MPC80 (◆) and lactose (▲).

$a_w$ = 0.32 is in more rapidly exchanging forms at hence more readily detected.

Hargreaves (1995) observed a decrease in spin-lattice ($T_1$) relation time with increasing $a_w$ up to $a_w$ = 0.382, but unfortunately did not report peak intensities. At $a_w$ = 0.43, where the lactose has crystallised, a considerable drop in intensity in this peak was observed. Water of crystallisation, which would be present in the crystalline lactose, has very short relaxation times and is not detected in low-field NMR measurements as used in this study. At $a_w$ up to 0.323, a similar peak in the $T_2$-range of $10^4$–$10^6$ ms was observed for SMP, MPC50 and MPC80 (Fig. 3). In addition, however, a notable peak was observed in the $T_2$-range of $10^1$–$10^3$ ms. The intensity of this peak increased with concomitant rise in protein content of the powders (SMP < MPC50 < MPC80) and was directly related to increasing $a_w$ (Fig. 3). In this $a_w$ range mono- and bilayer water and some multilayer water dominate (Chuy & Labuza, 1994), which explains the short relaxation times. With increasing $a_w$ >0.4, progressive further increases in peak area as well as shifts of the peaks towards longer relaxation times were observed (Fig. 3).

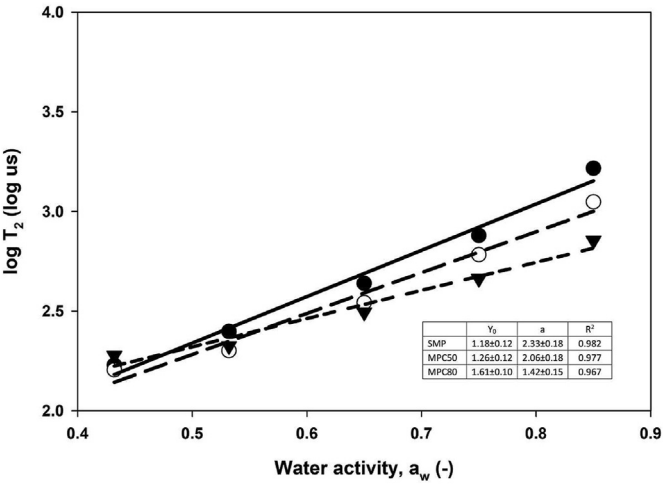


**Fig. 5.** Derived parameters of linear relationship between log $T_2$ relaxation time (log μs) and water activity ($a_w$) above 0.4 for SMP (●; fit, ——), MPC50 (○; fit, - - - -) and MPC80 (▼; fit, - - - -).

For both peak position and peak area, log-linear increases with increasing $a_w$ were observed (Fig. 4). Interestingly, however, the slope of such curves was highest for SMP, followed by MPC50 and MPC80 (Fig. 5), whereas based on the protein content, the opposite may have been expected, given that the lactose in the samples has crystallised and would not contribute. However, the SMP also contains a notable amount of soluble salts, which are present at smaller amounts in MPC50 and almost absent in MPC80 (Carić, 2002). Particularly at $a_w$ >0.5, it has been shown that the soluble salts fraction of milk powder can contribute very strongly to moisture sorption, binding in excess of 1 g of water per g of dry matter at $a_w$ >0.8 (Berlin, Anderson, & Pallansch, 1968). This is notably higher than water binding by MPC80 (Fig. 1). Hence, the higher peak areas for SMP compared with MPC50 and MPC80 in the $T_2$ relaxation profiles (Fig. 4) may be related to the contribution of the soluble milk salts to water sorption of the powders. Shifts to larger $T_2$ relaxation times with increasing $a_w$ can be related to more loosely bound multilayer and capillary water arising at higher water activity (Modig et al., 2004). In all cases, $T_2$ values remained 3 orders of magnitude lower than for free water, indicating that notable limitations in mobility remained.

## 4. Conclusions

Water mobility was studied in lactose, SMP, MPC50 and MPC80 as a function of $a_w$ by low-field NMR spectroscopy. Water associated with the protein phase and the amorphous lactose phase could be distinguished based on $T_2$ relaxation times. Log-linear increases in $T_2$ peak area with increasing $a_w$ were observed at $a_w$ >0.4, with strongest shifts found for SMP. These are likely related to the notable contribution of soluble milk salts to moisture sorption behaviour of SMP and related products. Overall, low-field NMR proved to be a very useful technique to study moisture sorption of dairy powders, providing complementary information next to sorption isotherms. Because the relaxation times are also measured, it is possibly to readily obtain insights in water relaxation and hence the propensity of water in the product to contribute to physical or chemical instability of the product.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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