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Water Properties in Cream Cheeses with Variations in pH, Fat, and Salt Content and Correlation to Microbial Survival

Sandie M. Møller,*[,†] Tina B. Hansen,[§] Ulf Andersen,[#] Søren K. Lillevang,[#] Anitha Rasmussen,[#] and Hanne C. Bertram[†]

[†]Department of Food Science, Faculty of Science and Technology, Aarhus University, Kirstinebjergvej 10, DK-5792, Årslev, Denmark

[§]DTU Food, National Food Institute, Division of Microbiology and Risk Assessment, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860, Søborg, Denmark

[#]Arla Strategic Innovation Centre, Arla Foods, DK-8220 Brabrand, Denmark

ABSTRACT: Water mobility and distribution in cream cheeses with variations in fat (4, 15, and 26%), added salt (0, 0.625, and 1.25%), and pH (4.2, 4.7, and 5.2) were studied using ^{1}H NMR relaxometry. The cheese samples were inoculated with a mixture of *Listeria innocua*, *Escherichia coli* O157 and *Staphylococcus aureus*, and partial least-squares regression revealed that ^{1}H T_2 relaxation decay data were able to explain a large part of the variation in the survival of *E. coli* O157 (64−83%). However, the predictions of *L. innocua* and *S. aureus* survival were strongly dependent on the fat/water content of the samples. Consequently, the present results indicate that NMR relaxometry is a promising technique for predicting the survival of these bacteria; however, the characteristics of the sample matrix are substantial.

KEYWORDS: *cream cheese, transverse relaxation, low-field NMR, water self-diffusion, Listeria innocua, Staphylococcus aureus, E. coli O157*

INTRODUCTION

Pathogenic bacteria are unwanted in food, and extensive efforts are made to prevent the presence of these bacteria. However, from time to time, pathogenic bacteria are detected in different foods, and it is therefore of interest to make sure that these bacteria cannot grow to such a number that they pose a threat to human health.

Since 1953 when Scott established a correlation between water activity and bacterial growth, overall water activity in food items has been used as an indicator of whether bacterial growth is possible.[1] However, a theory proposed by Hills et al.[2] suggests that the microstructure in food gives rise to different local water activities within the food. This theory was confirmed for a variety of model porous systems, proposing a new model for water activity, whereby the microstructure of the food should be taken into account when an overall water activity is calculated.[2] This theory is based on fast exchange of water between bulk, surface, and bound states, and the form of the theory is similar to that describing NMR water relaxation rates in the fast-exchange limit.[3] ^{1}H NMR T_2 relaxation can be used to investigate the availability of water in foods, where each state of water present has its own intrinsic water relaxation time. In cheese systems NMR relaxometry has been applied for monitoring changes in water mobility and water distribution during production of mozzarella and imitation cheese[4,5] and to characterize fat and water states in cheese.[6,7] Furthermore, NMR relaxometry has in dairy products been applied for the prediction of creaminess in cream cheese,[8] to characterize acidified milk drinks,[9] for the estimation of water-holding capacity in fresh cheese,[10] and to investigate the initial stage of cheesemaking, gel formation, gels, and syneresis development.[11−14]

Few studies have attempted to use NMR relaxometry to investigate the growth and survival of different pathogenic bacteria.[3,15,16] Hills et al.[3] found that in the slow, diffusive limit of NMR relaxation, it was possible to monitor the microscopic redistribution of water between different pores in the beds of porous media, and these changes were correlated with microbial recovery rates. In addition, a recent study by Møller et al.[15] found that 90% of the variation in the reduction of *Salmonella* and *Escherichia coli* VTEC in fermented sausages could be explained by NMR T_2 relaxation data. These findings are intriguing and reveal that NMR relaxometry contains information of importance for bacterial growth potential in foods.

The objective of the present study was (i) to investigate the mobility and distribution of water in a model system consisting of different cream cheeses using ^{1}H NMR relaxometry, (ii) to monitor the behavior of *Listeria innocua*, *Escherichia coli* O157, and *Staphylococcus aureus* over a period of 6 months and calculate survival parameters, and (iii) to investigate if any correlation could be established between the ^{1}H NMR relaxometry data and survival parameters of *L. innocua*, *E. coli* O157, and *S. aureus*, respectively.

MATERIALS AND METHODS

Microorganisms and Inoculum Preparation. *L. innocua* (DSM 20649), *E. coli* O157 (ATCC 43888), and *S. aureus* (ATCC 6538) were maintained as frozen suspensions at −80 °C. Cultures were grown in tryptone soy broth (Oxoid CM 0129, Basingstoke, U.K.) at

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Table 1. Sample Codes for the 11 Cream Cheese Products, Experimental Design, and Analyzed Chemical Composition, pH, and Water Activity (a_w)

	experimental design			analyzed chemical composition						
sample	fat (%)	salt (%)	pH	fat (%)	protein (%)	dry matter (%)	water (%)	salt[a] (%)	pH	a_w
N1	4	0	4.2	4.0	12.8	21.8	78.5	0.17	4.17	0.997
N2	26	0	4.2	24.5	4.8	33.3	67.0	<0.15	4.19	0.996
N3	4	0	5.2	4.0	12.5	21.1	78.4	<0.15	5.16	0.997
N4	26	0	5.2	24.4	4.8	33.5	66.9	<0.15	5.26	0.997
N5	4	1.25	4.2	3.9	12.3	22.7	77.5	1.35	4.23	0.987
N6	26	1.25	4.2	25.4	4.8	34.4	65.2	1.23	4.21	0.987
N7	4	1.25	5.2	3.9	12.4	22.2	77.8	1.36	5.10	0.988
N8	26	1.25	5.2	24.1	4.9	34.1	65.9	1.21	5.20	0.986
N9	15	0.625	4.7	14.7	11.1	31.0	69.3	0.73	4.81	0.993
N10	15	0.625	4.7	14.4	11.1	31.0	69.2	0.71	4.80	0.992
N11	15	0.625	4.7	14.6	11.2	30.9	69.4	0.73	4.80	0.994

[a]Salt percentage is calculated as the salt in the product, not in the water phase.

37 °C for 48 h. During preparation of the cheeses, 0.1% of each culture was added.

Cream Cheese Production. Eleven different cream cheeses with variations in pH, salt, and fat content were produced in a pilot plant at Arla Foods, Brabrand, Denmark, as described by Janhøj et al.[17] A factorial design with triplicate center points was applied (Table 1).

The 11 different cream cheese products (N1−N11) were made as large batches. Six containers from each cream cheese product were tapped and cold-stored; five containers were used for NMR analyses, and one container was used for determination of chemical composition, a_w, and pH measurements. The remaining cream cheese mixture from each cream cheese product was inoculated with *L. innocua* (an apathogen bacteria resembling the growth parameters for *Listeria monocytogenes*), *E. coli* O157, and *S. aureus* to a level of 10^5−10^6 CFU/g in the final product. Each inoculated cream cheese product was tapped into four containers. Two were stored at 11.0 ± 0.22 °C and two at 16.6 ± 0.04 °C until microbial analysis.

Chemical Composition (Fat, Protein, Water, Dry Matter, and Salt Content). Fat content was determined using the Van Gulik principal (ISO norm 3433). For protein analysis the block digestion method (ISO norm 8968-3) was applied, using a Tecator digestion system and a Kjeltec system (Foss, Hilleroed, Denmark) for determination of the nitrogen content. From the nitrogen content the protein content was calculated using a nitrogen conversion factor of 6.38. Dry matter content was analyzed using a drying method (ISO norm 5534). Water content was calculated by subtracting the dry matter percentage from 100. Salt content was analyzed using a potentiometric titration method (ISO norm 5943). Sample sizes were 1.500 ± 0.001 g for analysis of fat content, 0.5−1.5 g for analysis of protein content, and 2−5 g for analysis of dry matter, water, and salt content. Chemical composition was analyzed in duplicates in one container of each cream cheese product.

Water Activity Measurements. Water activity (a_w) was determined in all samples at the day of production, using an AQUA Lab equipment model series 3 TE 01089852 B (AQUA Lab, Riverside, CA) and a reference temperature of 25 °C. Water activity was measured in one container of each cream cheese product. The top layer of the cheese was removed, and a sample was taken from the core. The samples were taken in duplicates, and each duplicate was analyzed three times.

pH Measurements. pH was measured in one container of each cream cheese product at the day of production, using a Knick pH-meter 766 Calimatic (Knick, Berlin, Germany) equipped with an Inlab Solids electrode (Mettler Toledo, Greifensee, Switzerland).

NMR T_2 Relaxation Measurements. Proton NMR T_2 relaxation measurements were performed on a Maran benchtop pulsed NMR analyzer (Resonance Instruments, Witney, U.K.) operating at 23.2 MHz and equipped with an 18 mm variable-temperature probe, using a Carr−Purcell−Meiboom−Gill sequence.[18,19] The acquisition settings included a delay between the 90° and 180° pulses (τ spacing) of 150 μs, a recycle delay of 4 s, and the number of scans acquired (16). Samples (approximately 4 cm long and 1 cm in diameter) were analyzed at 25 °C. The T_2 relaxation was analyzed in samples from five containers of each cream cheese product (corresponding to a total of 5 × 11 = 55 measurements) one day after production.

The obtained T_2 decay data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler, Reeds, and Dawson,[20] and carried out in MatLab (The Mathworks Inc., Natick, MA) version 6.5 using in-house scripts. Distributed exponential fitting results were given in a plot of relaxation amplitude versus relaxation time over a predefined range of characteristic relaxation times. In this study we fitted 256 logarithmically distributed relaxation times from 0.5 to 3000 ms. Mean values (relaxation times) and the areas of the relaxation populations found were calculated.

NMR Water Self-Diffusion Coefficients. NMR water self-diffusion coefficients measurements were carried out using a Maran benchtop pulsed NMR analyzer (Resonance Instruments) operating at 23.2 MHz and equipped with an 18 mm variable-temperature probe and a gradient amplifier at a temperature of 25 °C, applying a pulse gradient spin echo sequence. The water self-diffusion was analyzed in samples from five containers of each cream cheese product (corresponding to a total of 5 × 11 = 55 measurements) one day after production. For each measurement, a total of 22 echoes were acquired as a function of the gradient pulse duration (δ), which was varied from 200 to 2500 μs, using a recycle delay of 2 s. The gradient pulse amplitude (g) was 5000, and the gradient pulse separation (Δ) was set to 200 ms. The diffusion coefficient was obtained by plotting

$$\log(\text{echo amp}) \text{ vs } \left((\gamma \times g)^2 \times \delta^2 \times \left(\Delta - \frac{\delta}{3}\right)\right)$$

where γ is 267538030 s^{-1} T^{-1}, and the diffusion coefficient was obtained as the negative slope of the best straight line fitted to this graph.

Microbial Analysis. Microbiological analyses on cream cheese samples were conducted after storage at 11.0 and 16.6 °C for 0, 2, 4, 5, 7, 8, 9, 12, 14, 16, and 26 weeks. Samples were taken from the core of the cheese to avoid surface molds. Cone-shaped cuts through the surface of the cheeses were performed with a narrow, sterile spoon. The surface was discarded, and approximately 5 g of cheese curd was aseptically transferred to filter bags, diluted 10-fold in maximum recovery diluent (MRD; Oxoid CM733, Hampshire, U.K.), and homogenized in a stomacher (stomacher 400, Seward, London, U.K.) at high speed for 2 min, before further 10-fold serial dilution in MRD. A combination of drop-plating of three 10 μL drops, surface spread-plating of 0.1 mL, and surface spread-plating of 3 × 0.333 mL of appropriate dilutions was performed for *L. innocua* and *E. coli* O157. For enumeration of *L. innocua*, Listeria selective agar (Oxoid CM0856) + Listeria selective supplement (Oxoid SR0206E),

incubated aerobically at 30 °C for 2 days, was used. For enumeration of *E. coli* O157, sorbitol MacConkey agar (Oxoid CM0813) + CT supplement (Oxoid SR0172E), incubated aerobically at 37 °C for 24−48 h, was used. The enumeration of *S. aureus* was performed using 1 mL of the appropriate 10-fold dilutions on 3 M petrifilm, Staph Express Count Plate (3 M Microbiology Products, St. Paul, MN), and aerobic incubation at 37 °C for 24−48 h. All growth data from plate counts were $\log_{10}$ transformed.

Data Analysis. Bacterial survival curves for *E. coli* O157 and *S. aureus* were fitted to a biphasic survival model with a shoulder using the Web edition of DMFit (http://modelling.combase.cc/DMFit.aspx). This procedure provided estimates of initial value ($\log_{10}$ CFU g^{-1}), shoulder length (days), and maximum inactivation rate. As survival curves for *L. innocua* showed signs of neither shoulder nor asymptote, a linear survival model was applied, resulting in estimates of initial value ($\log_{10}$ CFU g^{-1}) and maximum inactivation rate ($\log_{10}$ CFU day^{-1}). Shoulder length and maximum inactivation rate were used as response variables in further modeling.

Principal component analysis (PCA) and partial least-squares (PLS) regression models were performed using Simca-P+ software (Umetrics AB, Kinnelon, NJ). PCA was carried out on T_2 relaxation decay curves normalized according to sample weight to identify cluster patterns, whereas PLS regressions were carried out on T_2 relaxation decay curves normalized according to sample weight for prediction of survival of pathogens. From the PLS models, validated correlation coefficients (R^2) and root-mean-square error of cross-validation (RMSECV) were obtained for assessing how well it fitted. The cross-validation applied divided the samples in groups according to cream cheese product number when samples from one of the fat levels was studied, and segmented cross-validation divided the samples according to fat level when samples from more than one fat level were studied. For testing correlations between two single variables, simple linear regression was performed in Microsoft Excel 2010 (Microsoft, Redmond, WA). In addition, a Student *t* test was performed using SAS 9.1.3 software (SAS Institute Inc., Cary, NC).

RESULTS

NMR Relaxometry and Water Self-Diffusion Results. PCA of the normalized T_2 relaxation decays revealed a separation between samples with 26% fat and samples with 4 or 15% fat along principal component 1 (Figure 1). In addition, a separation between the pH levels and the salt levels was also observed (Figure 1), especially in cheese samples with 4% fat, for which the separation between the salt and pH levels was evident. Samples with 4% fat and pH 4.2 and samples with 15% fat and pH 4.7 were located very close together in three clusters. However, the placement of the clusters could indicate that only small differences are present in the T_2 relaxation decays between these samples. Distributed analysis of the T_2 relaxation data revealed three populations of protons in the cheese samples, and the distributed relaxation times for samples N1, N2, and N9 are shown in Figure 2. The three populations with T_2 relaxation times around 1−4, 8−31, and 75−190 ms are referred to as T_{21}, T_{22}, and T_{23} and represent 1.3−5.2, 0.7−15.6, and 80.7−97.2% of the total signal amplitude, respectively.

Determination of the T_2 relaxation time constants and relative areas (population sizes) for populations T_{22} and T_{23}, obtained by distributed exponential fitting, revealed considerable effects of fat content on both T_2 time constants as well as T_2 population sizes (Table 2). Cream cheeses with a fat content of 26% were characterized by longer T_{22} and T_{23} relaxation times, and a higher percentage of the protons was located in the T_{22} population compared to samples containing 4 and 15% fat. pH had a similar effect as fat content on the relaxation times and population size of T_{22} and T_{23}, as increases in the relaxation time of the two populations and in the relative area of T_{22} were

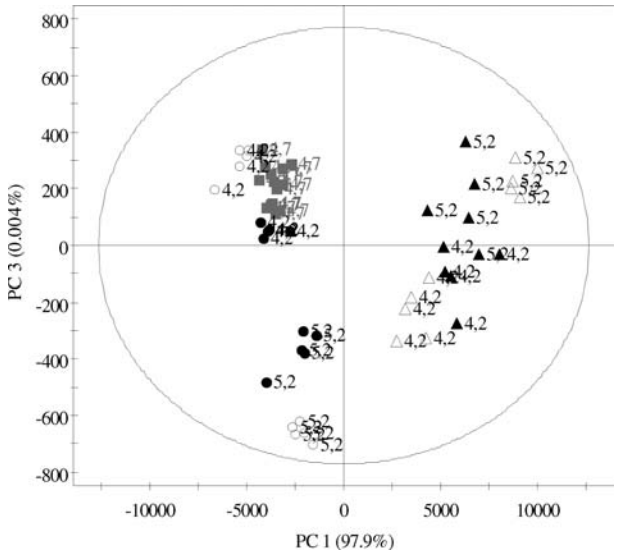

Figure 1. Principal component analysis plot calculated from normalized T_2 relaxation decay data, labeled according to pH level (4.2, 4.7, or 5.2): (grey square) 15% fat, 0.625% salt; (open circle) 4% fat, 1.25% salt; (solid circle) 4% fat, 0% salt; (open triangle) 26% fat, 1.25% salt; (solid triangle) 26% fat, 0% salt.

observed with increasing pH (Table 2). Salt content had no effect on the calculated T_2 time constants or T_2 population sizes (data not shown).

The water self-diffusion coefficient was analyzed in all samples, which resulted in coefficients in the magnitude of $8.36E^{-10}$−$1.10E^{-9}$ m^2/s. In Figure 3, the water self-diffusion coefficients are plotted against water content. A separation into two groups is observed, revealing that samples N1, N3, N5, and N7 with water contents of 77.45−78.53% had a significantly higher water self-diffusion coefficient compared to the samples with water contents in the range of 65.17−69.18%.

Chemical Composition, Water Activity, and pH. Chemical composition, pH, and water activity were analyzed (Table 1). Three levels of fat, salt, and pH were identified according to cheese recipes. In addition, three levels of protein, dry matter, and water content were obtained, together with three levels of water activity (Table 1).

Microbiological Results. The counts of *L. innocua*, *E. coli* O157, and *S. aureus* were measured several times over a period of 6 months at 11.0 and 16.6 °C. In a few of the samples (N7 and N8), detection of the bacteria was possible until 110 days of storage at 11.0 °C. However, in most of the samples, the bacterial level reached a level that was not detectable within the first 2 months of storage. In these cases survival was estimated from the measurements completed. None of the samples revealed growth of the bacteria in the cheeses. Instead, a decrease in the level of the bacteria was observed. In N1−N6, a rapid decrease of *L. innocua* from 10^5−10^6 to 10^3−10^4 CFU g^{-1} was observed immediately after production. This decrease was most pronounced for the cream cheeses having pH around 4.2. Following this rapid decrease, survival of *L. innocua* continued with a log−linear decay. Maximum inactivation rates were estimated from survival curves, and N1−N6 showed the fastest reduction with approximately −0.3 $\log_{10}$CFU day^{-1} at 11.0 and 16.6 °C (Table 3). The slowest inactivation was observed in N7 and N8 with pH of approximately 5.2 and 1.8% salt in the water phase. In those cream cheeses, −0.02 and −0.04 $\log_{10}$CFU day^{-1} were observed at 11.0 and 16.6 °C, respectively (Table

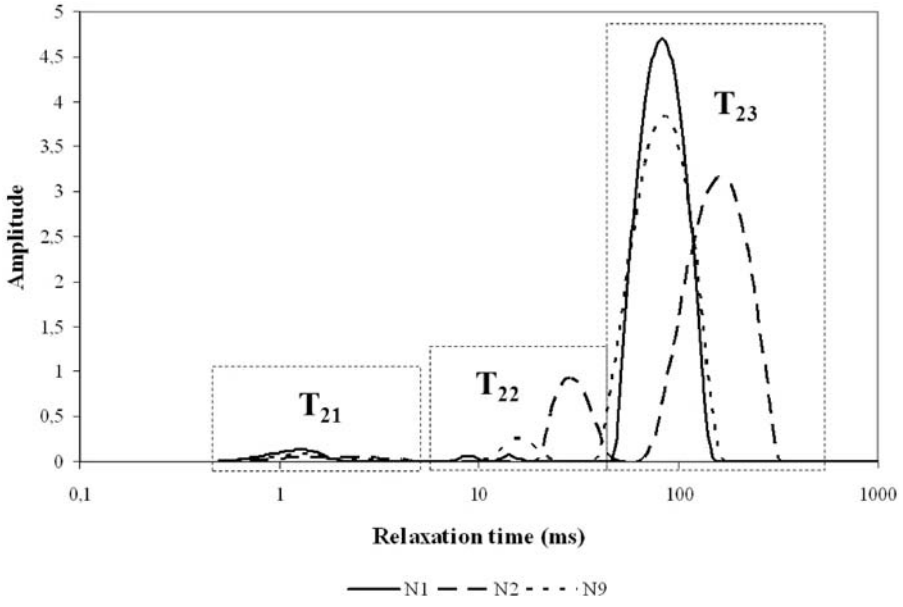

Figure 2. Distributed relaxation times, average of five T_2 relaxation measurements, for samples (solid line) N1 (4% fat, 0% salt, and pH 4.2), (long-dash line) N2 (26% fat, 0% salt, and pH 4.2), and (short-dash line) N9 (15% fat, 0.625% salt, and pH 4.7). The boxes frame the three different populations (T_{21}, T_{22}, and T_{23}).

Table 2. Relaxation Time and Corresponding Relative Area Obtained from Distributed Exponential Fitting of ^{1}H NMR T_2 Relaxation Decays, Obtained from 11 Cream Cheese Products

sample	T_{21} (ms)	area T_{21}	T_{22} (ms)	area T_{22}	T_{23} (ms)	area T_{23}
N1	2.8	1.3	11.1	0.9	82.3	96.1
N2	2.1	2.6	28.8	14.0	162.9	83.9
N3	3.7	1.4	15.7	1.9	96.3	94.3
N4	2.2	2.7	30.0	15.1	181.7	82.6
N5	2.6	1.3	12.1	1.0	77.9	96.3
N6	2.4	1.6	27.7	13.4	155.3	84.2
N7	4.5	1.4	15.6	2.1	101.7	93.4
N8	2.9	2.2	30.0	15.3	189.2	81.8
N9	2.3	1.6	15.8	3.3	84.6	94.3
N10	2.5	1.6	14.8	3.0	84.0	94.5
N11	2.5	1.4	14.5	3.0	82.3	94.4

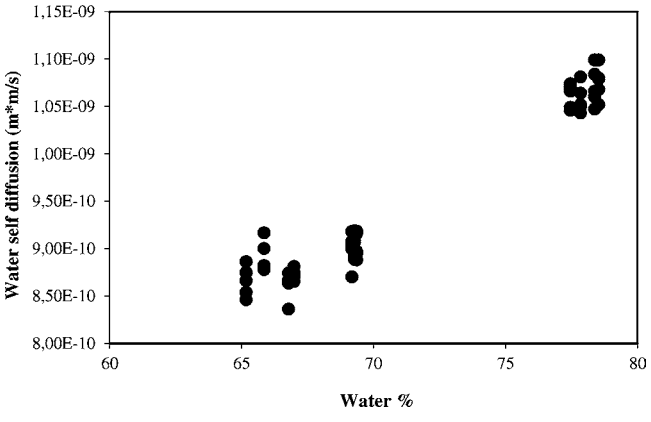

Figure 3. Water self-diffusion coefficient as a function of water content, analyzed in five containers of each cream cheese product.

Table 3. Mean Maximum Inactivation and Shoulder of *Listeria innocua*, *Escherichia coli* O157, or *Staphylococcus aureus* Obtained at 11.0 and 16.6 °C, Obtained for 11 Independent Cream Cheese Products Analyzed in Duplicate

		max inactivation rate (log_{10}CFU day^{-1})			shoulder (days)	
storage temp (°C)	sample	*L. innocua*	*E. coli* O157	*S. aureus*	*E. coli* O157	*S. aureus*
11.0	N1	−0.25	−0.25	−0.35	0.0	0.0
	N2	−0.27	−0.27	−0.37	0.0	0.0
	N3	−0.24	−0.11	−0.07	28.9	44.4
	N4	−0.23	−0.08	−0.12	30.6	43.3
	N5	−0.25	−0.25	−0.29	0.0	0.0
	N6	−0.27	−0.27	−0.32	0.0	0.0
	N7	−0.02	−0.03	−0.06	42.2	25.0
	N8	−0.02	−0.03	−0.09	63.4	39.8
	N9	−0.07	−0.12	−0.13	25.5	2.2
	N10	−0.09	−0.15	−0.10	32.1	0.0
	N11	−0.12	−0.11	−0.09	24.5	0.0
16.6	N1	−0.29	−0.29	−0.31	0.0	0.0
	N2	−0.32	−0.32	−0.35	0.0	0.0
	N3	−0.29	−0.08	−0.18	20.1	34.1
	N4	−0.20	−0.13	−0.19	23.3	31.0
	N5	−0.29	−0.29	−0.32	0.0	0.0
	N6	−0.32	−0.32	−0.15	0.0	0.0
	N7	−0.04	−0.10	−0.18	44.1	32.4
	N8	−0.03	−0.07	−0.33	51.7	77.8
	N9	−0.13	−0.21	−0.24	16.0	8.0
	N10	−0.14	−0.11	−0.15	20.8	3.2
	N11	−0.15	−0.13	−0.09	14.6	0.0

3). A rapid decrease immediately after production was also observed for *E. coli* O157 in N1−N6, but for *S. aureus* this was observed in only N1. For *E. coli* O157 and *S. aureus*, survival curves typically had a shoulder before a log−linear decrease began (Figure 4). The shoulder of *E. coli* O157 primarily depended on pH, with decreasing pH resulting in shorter shoulder length and ending at 0 days for pH around 4.2 (N1, N2, N4, and N5). At the highest pH level, 5.1−5.3, the shoulder length varied between 20.1 and 63.4 days depending

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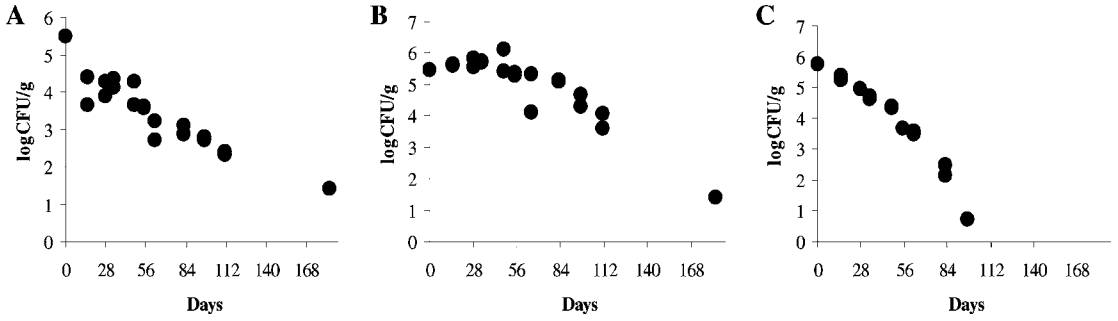

Figure 4. Log_{10}CFU/g (A) *Listeria innocua*, (B) *Escherichia coli* O157, and (C) *Staphylococcus aureus* as a function of days for sample N7 (pH 5.2, 4% fat, and 1.25% salt) stored at 11.0 °C.

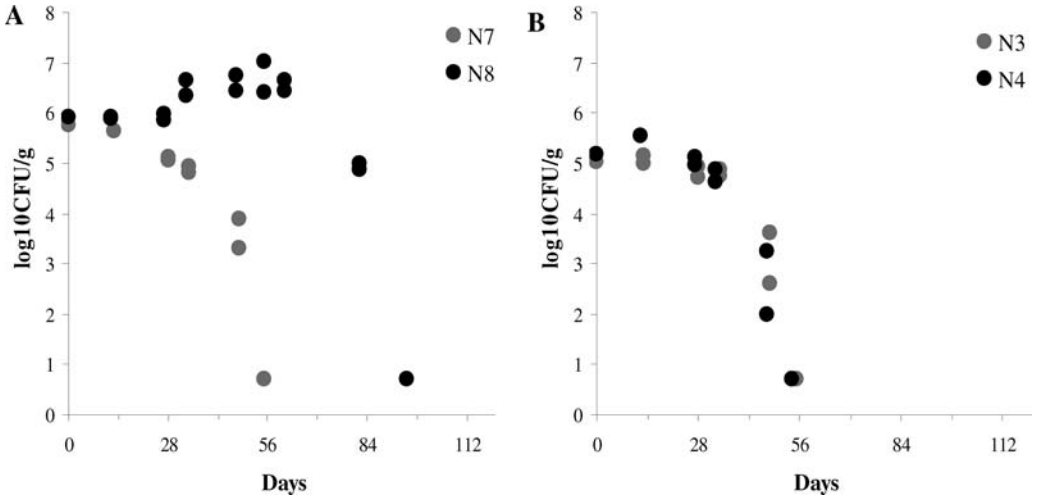

Figure 5. Log_{10}CFU/g *Staphylococcus aureus* as a function of days: (A) cream cheese types N7 (pH 5.2, 4% fat, and 1.25% salt) and N8 (pH 5.2, 26% fat, and 1.25% salt); (B) cream cheese types N3 (pH 5.2, 4% fat, and 0% salt) and N8 (pH 5.2, 26% fat, and 0% salt), stored at 16.6 °C.

on salt content, the highest level (1.8% in the water phase) giving rise to the longest shoulders (Table 3). For *S. aureus*, the shoulder length depended on pH in the same way as observed for *E. coli* O157. However, neither salt nor temperature affected the length of the shoulder for *S. aureus*. In regard to maximum inactivation rates, the fastest reduction of *E. coli* O157 as well as *S. aureus* was found in the cream cheeses N1, N2, N5, and N6 with pH 4.2, for which approximately −0.3 to −0.4 log_{10}CFU day^{-1} was observed (Table 3). Maximum inactivation rates decreased linearly with increasing pH for *E. coli* O157 at 11.0 and 16.6 °C and for *S. aureus* at 11.0 °C. At 16.6 °C, maximum inactivation rates of *S. aureus* were slowest in cream cheeses N9 to N11 with pH 4.8, and in the cream cheeses with pH 5.1−5.3 (N3, N4, N7, and N8), an interaction was observed between salt and water/fat content. In the cream cheese samples with no added salt, maximum inactivation rates were −0.18 log_{10}CFU day^{-1} independent of water/fat content, whereas in cream cheeses with 1.7−1.8% salt in the water phase, the maximum inactivation rate was 2-fold faster in N8 with the lowest water content than in N7 (Table 3 and Figure 5).

Prediction of Chemical and Microbiological Parameters. PLS regression analysis and linear regression were used to elucidate correlations between the ^{1}H NMR parameters (normalized T_2 relaxometry decay curves, distributed T_2 relaxation parameters, and water self-diffusion coefficients), chemical composition data, shoulder length, and maximum inactivation rate data for the three bacteria.

The normalized ^{1}H T_2 relaxometry decay data for all of the samples revealed a strong correlation to protein (R^2 = 0.86), whereas the correlation to fat was weaker (R^2 = 0.58), and even weaker for correlations to water content and dry matter content were observed (R^2 = 0.29 and 0.27, respectively). Probing the correlations between the distributed ^{1}H T_2 relaxometry parameters and data on chemical composition revealed correlation coefficients with different strengths. Strong correlations between the relaxation time of populations T_{22} and T_{23} and population sizes of T_{22} and T_{23} on the one hand and fat and protein content on the other hand were established, whereas the correlation to water and dry matter content was more moderate (Table 4). Furthermore, strong correlations were observed between the water self-diffusion coefficient and water content (R^2 = 0.95), fat content (R^2 = 0.81), and dry matter content (R^2 = 0.95).

Linear regression analysis revealed strong correlations between pH and some of the microbiological survival data (Table 5); however, only very weak correlations could be established between water activity and some of the microbiological survival parameters (Table 5).

PLS regression analysis of the normalized T_2 relaxation decays and the microbiological shoulder and maximum inactivation rate data including all cheese samples, calculated using segmented cross-validation according to fat level, revealed no correlations to survival of *L. innocua*, *E. coli* O157, or *S. aureus* or shoulder length of *E. coli* O157. However, a weak correlation to shoulder length of *S. aureus* (R^2 = 0.23) and a

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Table 4. Linear Regression Results Using Relative Area or Relaxation Time for Populations T_{22} and T_{23} as *x*-Variable, Obtained on Five Replicates from Five Different Containers, and Mean Values for Water Content, Fat Content, Protein Content, or Dry Matter Content as Response Variable, Obtained on 11 Independent Types of Cream Cheese Products

x-variable	response variable	*N*	R^2
relative area T_{22}	water %	11	0.65
	protein %	11	0.99
	fat %	11	0.84
	dry matter %	11	0.63
relaxation time T_{22}	water %	11	0.56
	protein %	11	0.90
	fat %	11	0.75
	dry matter %	11	0.54
relative area T_{23}	water %	11	0.57
	protein %	11	0.95
	fat %	11	0.77
	dry matter %	11	0.55
relaxation time T_{23}	water %	11	0.46
	protein %	11	0.90
	fat %	11	0.67
	dry matter %	11	0.44

Table 5. Linear Regression Results, Using Analyzed Mean pH or Mean Water Activity (a_w) as *x*-Variables and the Shoulder or Maximum Inactivation Rate of *Listeria innocua*, *Escherichi coli* O157, or *Staphylococus aureus* as Response Variables, Obtained for 11 Independent Cream Cheese Products Analyzed in Duplicate from Two Different Containers of Cheese

storage temp (°C)	microorganism	response variable	*N*	pH R^2	a_w R^2
11.0	*L. innocua*	max inactivation rate ($\log_{10}$ CFU day^{-1})	11	0.34	0.20
	E. coli O157	max inactivation rate ($\log_{10}$ CFU day^{-1})	11	0.88	0.02
		shoulder (days)	11	0.76	0.07
	S. aureus	max inactivation rate ($\log_{10}$ CFU day^{-1})	11	0.89	0.00
		shoulder (days)	11	0.61	0.02
16.6	*L. innocua*	max inactivation rate ($\log_{10}$ CFU day^{-1})	11	0.53	0.12
	E. coli O157	max inactivation rate ($\log_{10}$ CFU day^{-1})	11	0.85	0.00
		shoulder (days)	11	0.71	0.11
	S. aureus	max inactivation rate ($\log_{10}$ CFU day^{-1})	11	0.12	0.00
		shoulder (days)	11	0.55	0.06

very weak correlation to shoulder length for *E. coli* O157 (R^2 = 0.06) were obtained. Excluding the samples containing 15% fat, thereby including only the data for cheese samples with 4 and 26% fat in the PLS regression analysis, resulted in very weak correlations to maximum inactivation rate of *L. innocua* (R^2 = 0.03), maximum inactivation rate of *E. coli* O157 (R^2 = 0.03), shoulder for *E. coli* O157 (R^2 = 0.12), and shoulder for *S. aureus* (R^2 = 0.14). However, no correlation to the maximum inactivation rate of *S. aureus* was obtained. In the search for even better correlations, the contribution from fat was eliminated by dividing the samples into the different fat levels, 4, 26, and 15% fat, respectively, and subsequently the correlations between the normalized T_2 relaxometry decay curves and the microbiological survival data were elucidated. Samples N9, N10, and N11 (15% fat) were all produced to have identical fat, pH, and salt levels, and consequently no correlations to the microbiological results could be obtained. Correlations between normalized T_2 relaxometry decay curves and the shoulder lengths and maximum inactivation rates of the three bacteria are shown in Table 6 for each of the fat levels separately. In cheese samples with 4% fat, moderate−strong correlations to shoulder length and maximum inactivation rate of both *E. coli* O157 and *S. aureus* were obtained. Similar correlations were observed in cheese samples with 26% fat; however, no correlation between T_2 relaxation decay data and maximum inactivation rate of *S. aureus* was obtained in cheese samples with 26% fat. In contrast, a moderate correlation to maximum inactivation rate of *L. innocua* was obtained. Including the temperatures at which the microbiological analyses were performed as an *x*-variable resulted in similar correlations (data not shown). The predicted maximum inactivation rate for *E. coli* O157 versus the actual analyzed value in cheese samples with 26% fat is shown in Figure 6.

DISCUSSION

In the present study, 11 independent cream cheese products were produced and subjected to ^{1}H T_2 relaxometry and water self-diffusion coefficient measurements and analysis of the chemical composition, pH, and water activity. In addition, two sets of samples were inoculated with *L. innocua*, *E. coli* O157, and *S. aureus*, and the survival of these bacteria was monitored over 6 months at two different temperatures, 11.0 and 16.6 °C.

Distributed analysis of the ^{1}H T_2 relaxometry data revealed the presence of three populations of protons in the cream cheese samples, T_{21} (1−4 ms), T_{22} (8−31 ms), and T_{23} (75−190 ms), respectively. As the cream cheese samples had a fat content up to 25%, contribution from fat protons to the three populations can be expected, even though water is the main constituent. An earlier ^{1}H NMR relaxation study on cream cheese with a fat content of 26.5% revealed four populations of protons when nonsheared cream cheese was analyzed at 68 °C: an immobilized/solid population (0.009 ms), a weak mobile population (0.5 ms), a moderate mobile population (60−90 ms), and a very mobile population (200−330 ms).[10] In the present study, the T_{21} population with a relaxation time of 1−4 ms can be ascribed to bound/weak mobile protons, the T_{22} population with a relaxation time of 8−31 ms can be ascribed to moderate mobile protons, and the main population T_{23}, representing 80.7−97.2% of the protons present, can be ascribed to mobile protons in the cream cheese samples. Andersen et al.[8] studied ^{1}H T_2 relaxation in low-fat cream cheese samples with variations in fat (0−9%), added salt (0.4−0.9%), and pH (4.4−5.0) using low-field NMR relaxometry. A two-component, discrete exponential fitting of T_2 data resulted in T_2 relaxation populations in the ranges of 30−62 ms (T_{21}) and 87−172 ms (T_{22}),[8] the latter being similar to the relaxation time of the T_{23} population in the present study. In the present study, fat content had a significant effect on the relaxation time and the relative area of the T_{22} and T_{23} populations (Table 2). A longer relaxation time for T_{22} and T_{23}, a larger relative area of T_{22}, and a smaller relative area of T_{23} were observed in cream

Table 6. Partial Least-Squares Regression Analysis Results Calculated Using the Normalized T_2 Relaxation Decay Data as *x*-Variables (n = 2048) Obtained from Four Different Cream Cheese Products Analyzed in Five Replicates from Five Different Containers and the Mean Value of Microbial Survival Parameters Obtained from Four Different Cream Cheese Products at 11.0 and 16.6 °C as Response Variable, Obtained from Samples with a Fat Level of 4 or 26%[a]

		fat level 4%			fat level 26%		
microorganism	response variable	PC[b]	R^2	RMSECV	PC[b]	R^2	RMSECV
Listeria innocua	max inactivation rate (log_{10}CFU day^{-1})	0			2	0.49	0.09
Escherichia coli O157	max inactivation rate (log_{10}CFU day^{-1})	2	0.78	0.05	2	0.83	0.05
	shoulder (days)	2	0.64	11.43	2	0.69	13.68
Staphylococcus aureus	max inactivation rate (log_{10}CFU day^{-1})	2	0.61	0.07	0		
	shoulder (days)	2	0.76	9.31	2	0.81	11.18

[a]The PLS regression analysis was calculated from 8 observations (4 cream cheese products × 2 temperatures), cross-validated according to cheese product (cross validation groups = 4). [b]PC, number of principal components.

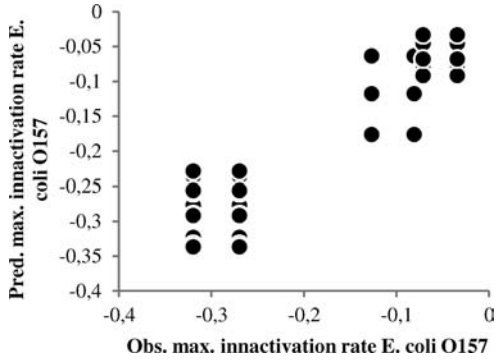

Figure 6. Observed versus predicted maximum inactivation rate of *E. coli* O157 obtained from cream cheese samples with a fat level of 26%. The predicted values are calculated from partial least-squares regression analysis using normalized T_2 relaxation decay curves as *x*-variable (n = 2048). Five replicates from five different containers of four cream cheese products are included; the mean value of maximum inactivation rate for *E. coli* O157 obtained at 11 and 16.6 °C is used as response value, cross-validated according to cream cheese product.

cheese samples with approximately 26% fat compared with samples containing either 4 or 15% fat, revealing that both T_{22} and T_{23} contained contributions from fat. The effect of fat content in cream cheese samples was also studied by Andersen et al.,[8] who found that fat content had a significant effect on relaxation time and concentration of population T_{21} and T_{22}. The different fat levels were linked together with the different water and protein levels, where the low-fat cream cheese had a high protein content and higher water content and vice versa. In the present study, a moderate correlation was obtained between the relaxation time of T_{22}, relaxation time of T_{23}, or relative area of T_{22} on the one hand and water content on the other hand (Table 4). The variation in fat level was larger than the variation in water content, which could explain the superior correlations between the distributed T_2 relaxation time constants and the relative T_2 population sizes and fat content compared to water content. Even stronger correlations were established between the distributed T_2 relaxation times and relative areas of T_{22} and T_{23} and the protein levels, where the four different NMR relaxation parameters explained 90% or more of the variation in protein content (Table 4). The proteins together with the acidification and heat treatment are responsible for the formation of a gel when cream cheese is made, and the protein content has a substantial impact on the final microstructure of cream cheeses. Thus, the strong correlation between the T_2 relaxation data and the protein level shown in the present study extends this knowledge and reveals that the protein level mainly determines the variations in the distribution and the mobility of the protons present in the samples.

Diffusion measurements revealed two levels of water self-diffusion in the investigated cream cheese samples, which were related to differences in the water content (Figure 3). Samples with the highest water content (77.45−78.53% water), and hence the lowest dry matter content, displayed the highest water self-diffusion coefficient. Earlier studies have also observed effects of dry matter content on the water self-diffusion, revealing a decrease in water self-diffusion with increasing dry matter content.[21,22] In addition, Colsenet et al.[22] found an effect of solution/gel state when the whey protein concentration exceeded 20%. For concentrations above 20%, an increased reduction in water self-diffusion with increasing whey protein content was observed in gels compared to solutions.[22] Mtais et al.[21] observed that other factors such as caseins, fat globules, and soluble fractions also affected the water self-diffusion in complex dairy systems. However, in the present study the water content/dry matter content seemed to be the only factor affecting the water self-diffusion. Minor differences in the water self-diffusion coefficient were observed in the group with water contents between 65.2 and 69.4%, but these differences were numerically small and nonsignificant.

For all three bacteria, reductions during storage at 11.0 °C as well as 16.6 °C were observed. For *L. innocua*, an immediate reduction was seen, whereas *E. coli* O157 and *S. aureus* revealed a shoulder, with a constant level of bacteria before a reduction appeared (Figure 4). In theory, all three bacteria used in the present study are able to grow at the storage temperatures 11.0 and 16.6 °C if the intrinsic characteristics, such as salt content and pH, allow growth. Both *L. innocua* and *S. aureus* have been reported to be quite salt tolerant, and both *E. coli* O157 and *S. aureus* can grow down to water activities of 0.95 and 0.83, respectively.[23] The salt levels from <0.15 to 1.36% that were used in the present study correspond to salt in the water phase of approximately 0.2−1.8% and water activity between 0.987 and 0.997, respectively, and, therefore, represent conditions under which the applied bacteria still should be able to grow. The limiting factor affecting growth of the bacteria used in the present study seemed to be pH. *L. innocua* has been reported to grow down to a pH of 4.4, whereas growth of *E. coli* O157 and *S. aureus* has been observed down to pH 4.4 and 4, respectively, when all other conditions are optimal.[23] The cream cheese

samples had pH values between 4.17 and 5.26, which are far below optimal growth pH for all three bacteria. Hence, pH is expected to be the factor with largest impact on the behavior of *L. innocua*, *E. coli* O157, and *S. aureus* in cream cheese. This was confirmed by the fact that correlations between pH and many of the microbiological survival data were observed (Table 5).

The observed shoulder and maximum inactivation rate for *E. coli* O157 and *S. aureus* have to be linked together to predict the overall survival in cream cheese. Therefore, to use pH or ^{1}H NMR T_2 relaxation decay for prediction of survival potential of these two bacteria, high correlations between pH or ^{1}H NMR T_2 relaxation decay and both shoulder as well as maximum inactivation rate for these two bacteria would have to be established. Using pH as *x*-variable in PLS regression, shoulder as well as maximum inactivation rate, *E. coli* O157 were successfully predicted at both 11.0 and 16.6 °C, whereas for *S. aureus* this was only possible at 11.0 °C. At 16.6 °C, the shoulder length of *S. aureus* survival curves correlated well to pH, but the maximum inactivation rate did not (Table 5), as maximum inactivation rates at pH 5.2 were faster than at pH 4.8. In cream cheeses with pH 5.2, overall survival of *S. aureus* at 16.6 °C appeared to depend on the cheese composition, which entailed that the best survival was observed in the cream cheese with the highest fat (24.1%) and salt contents (approximately 1.8% in the water phase). Other studies have found that bacterial survival was improved in high-fat compared to low-fat foods.[24,25] One hypothesis is that fat provides a physical protection of the bacterial cell against organic acids and other antibacterial components.[25] Another possible explanation is that fat, like other food compounds, inhibits the antibacterial effect of these components.[24,26,27] As shown in Figure 5, the reason for the longer survival was a much longer shoulder. It even seemed that *S. aureus* was able to grow approximately 1 $\log_{10}$-unit from day 27 to day 55 in this particular cream cheese. Growing cells are usually more susceptible to inhibitory compounds than nongrowing cells, which may explain the subsequent, very rapid decrease observed for *S. aureus* in this cream cheese. For *L. innocua*, it was possible to establish a moderate correlation to the maximum inactivation rate using pH as *x*-variable at 16.6 °C. However, at 11.0 °C the correlation was weaker (Table 5).

Water activity was determined for all 11 cream cheese products, which revealed three levels of water activity in the range of 0.986−0.997 that corresponded to the three levels of salt in the samples. Equilibrium water activity coefficient (a_w) has been used as a general measure for water availability and is expressed as the ratio of the equilibrium vapor pressure of the system P to the vapor pressure of pure water at the same temperature P_0. However, foods are complex and heterogeneous systems, which are not necessarily at equilibrium at adsorption or desorption.[2] Hence, an overall water activity coefficient cannot always be used as an indicator of whether microorganisms can grow. This was noticed by Hills et al.,[3,28] who revealed that the survival of *S. typhimurium* did not correlate with the global, average water activity in different porous media. Consistent with these findings, the present study did not find any correlations between water activity and the shoulder and maximum inactivation rate for the three bacteria *L. innocua*, *E. coli* O157, and *S. aureus*, irrespective of temperature. This finding shows that in these cream cheese samples, water activity alone cannot provide information about the survival of these bacteria, probably due to the fact that the microstructure of the different cheese samples results in different local water activities in the product. The microstructure of foods depends among other things on the level of protein, fat, and salt, but also pH has an influence. The complex microstructure and different states of water present in foods mean that water activity can vary throughout the food system and is affected by changes in microstructure.[2] Hills et al.[28] suggested that physical methods sensitive to the air−water distribution and affected by microstructure, such as NMR water ^{1}H relaxation time distribution and electrical conductivity, may be more useful for predicting microbial survival in microporuos food matrices. Therefore, a better way of determining the availability of water in foods may be by using ^{1}H T_2 relaxometry, with which information about the distribution and mobility of the different states of water is obtained. An earlier study by Møller et al.[15] on fermented sausages produced with different starter cultures and different fermentation temperature revealed that NMR T_2 relaxometry data measured at the end of fermentation and before drying could explain about 90% of the variation in the reduction of *Salmonella* and *E. coli* VTEC. Hills et al.[3] also demonstrated a relationship between ^{1}H NMR relaxation and the recovery of *S. typhimurium*. Using different porous media as food model systems, Hills et al.[3] found that in the slow diffusive limit of NMR relaxation, it was possible to monitor the redistribution of water between the different pores in the beds, which could be correlated to microbial recovery rates. Also, studies by Vittadini et al.[16] and Lavoie et al.[29] are intriguing, as they have investigated the connection between NMR and microbial growth. Vittadini et al.[16] probed the correlation between microbial response of *S. aureus* and molecular mobility measured using ^{1}H and ^{2}H NMR relaxometry. They concluded that molecular mobility described by NMR relaxometry is a possible tool for describing water availability in solid and semisolid systems, such as dry and intermediate-moisture foods. However, in high-moisture, liquid, homogeneous systems, water activity was still found to be a valid indicator for *S. aureus* activity.[16] Furthermore, Lavoie et al.[29] found improved correlations between the mobility of water, determined using ^{17}O NMR relaxometry, and the growth rate of *S. aureus* in brain−heart infusion medium and concluded that the dynamic property of water molecules is an important determinant in the growth of *S. aureus*.[29] ^{17}O NMR relaxometry is a more direct measure of water mobility compared to ^{1}H NMR relaxometry, where protons from macromolecules (including fat) may contribute to the ^{1}H NMR relaxation. However, from the investigation by Lavoie et al.[29] ^{17}O NMR relaxometry does not seem to be superior to ^{1}H NMR relaxometry, as Lavoie et al.[29] were not able to establish any correlations between ^{17}O T_2 relaxation time and the microbial parameters, only between the ^{17}O NMR signal intensity and the microbial parameters. In addition, these studies by Vittadini et al.[16] and Lavoie et al.[29] investigated the connection between NMR and microbial growth in simpler systems and over a shorter period compared to the present study. Moreover, the present study differs from these two studies by investigating the ability of NMR relaxometry to predict the future microbial survival in cream cheese. This was done by investigating whether the T_2 relaxation decay curves obtained one day after production of the cream cheese samples could be used to predict the shoulder lengths and maximum inactivation rates of the three bacteria kept at 11.0 and 16.6 °C over 6 months. Including the T_2 relaxation decays for all 11 cream cheese products did not

reveal any significant correlations to the microbial survival parameters, and eliminating the samples with 15% fat resulted in only weak correlations (R^2 = 0.03−0.14). However, by dividing the samples according to their fat content, correlations to some of the shoulders and maximum inactivation rates of the three bacteria could be obtained (Table 6). Thus, in samples with 4% fat T_2 relaxation decay data were able to predict 64−78% of the variation in shoulder and maximum inactivation rate of *E. coli* O157, whereas in samples with 26% fat, the prediction was slightly better (69−83% explained variation). The T_2 relaxation decay curve could explain 49% of the variation in *L. innocua* maximum inactivation rates for samples with 26% fat. However, it was not possible to predict the maximum inactivation rate for *L. innocua* in samples with 4% fat. In addition, 61−76% of the variation of shoulder and maximum inactivation rate of *S. aureus* were explained in samples with 4% fat. However, it was not possible to establish a correlation to maximum inactivation rate of *S. aureus* for samples with 26% fat. Hence, T_2 relaxation decay data were not able to predict the survival of *S. aureus* for samples with 26% fat in the present study. Except for *S. aureus* in cream cheeses with high pH, survival of the three bacteria did not seem to be affected by the fat content (Table 3). However, the T_2 relaxation was affected by fat. The difference in fat content is linked with a difference in protein content; hence, the cream cheese samples with a fat level of 4% had a high protein content (12.30−12.79%) and cheese samples with a fat level of 26% had a low protein content (4.83−4.87%). This difference in protein content will affect the water−protein interaction due to extremely different microstructures, which could have an effect on the survival conditions for the bacteria present in the cream cheese samples. The correlations obtained were calculated using only eight data points for the microbial survival, which is far from enough data points to make a confident conclusion. However, even though the correlations between NMR variables and microbial survival parameters were moderate, the results were better than results obtained using water activity, which revealed only very weak correlations to the survival of the three bacteria (Table 5). Nevertheless, additional studies have to be made, including more samples and focusing on only one factor at a time, as several factors seem to cause conflicting effects on water mobility and distribution.

AUTHOR INFORMATION

Corresponding Author

*Phone: +45 8715 8305. Fax: +45 8715 4812. E-mail: Sandie.Moeller@agrsci.dk.

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