Journal of Dairy Research (2002) **69** 619–632. © *Proprietors of Journal of Dairy Research 2002*
DOI: 10.1017/S002202990200585X *Printed in the United Kingdom*

Moisture measurement in cheese analogue using stretched and multi-exponential models of the magnetic resonance T_2 relaxation curve

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(*Received 25 January 2001 and accepted for publication 28 April 2002*)

Summary. The dairy industry would benefit from rapid and non-destructive determination of moisture content of cheese products. The two components primarily responsible for the low-field magnetic resonance (MR) spin-spin relaxation (T_2) signal of cheese products are fat and the water bound to protein. If the moisture component of the signal can be distinguished from the fat component, it should be possible to measure moisture using an MR sensor. Therefore, a key aspect of the development of an MR moisture measurement method is examination of techniques for analysis of T_2 relaxation curves. One common method of T_2 analysis of complex foods, such as cheese, is to fit multi-term exponential models to the curves. An alternative approach is proposed which uses stretched exponential models. The single-term stretched exponential model has been used for porous rock systems and polymers, but not for foods. The T_2 relaxation curves were analysed using both models and the results were compared. The number of unknowns in the three-term exponential and two-term stretched exponential models was reduced by assuming the relaxation curve of the fat component was the same as the relaxation curve of pure fat. In each model, one of the exponential terms described the behaviour of the water in the cheese analogue, while the remaining term or terms described the behaviour of the fat. For each model the T_2 relaxation time associated with the water was well correlated with moisture content. Coefficients of determination of the relaxation time versus moisture from each of the two models were nearly identical. The advantages and disadvantages of the two models are discussed.

Keywords: Magnetic resonance, cheese, cheese analogue, moisture measurement, spin-spin (T_2) relaxation.

Proton magnetic resonance (MR) is an important tool for studying the properties of foods. Relaxation times determined with the MR sensor can reveal how tightly water molecules are bound to proteins and other components in food systems (Ruan & Chen, 1998). Spin-echo or spin-spin relaxation time (T_2) measurements are frequently used to monitor moisture in dairy products (Hester & Quine, 1976,

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1977; Schlesser *et al.* 1992; Brosio & Barbieri, 1996; Chaland *et al.* 2000). Since the moisture content of processed cheese products can vary widely and can also affect physical properties, it seems possible that an MR sensor could be used for rapid and non-destructive monitoring of moisture content and associated physical properties of cheese. An accurate and rapid method of measuring moisture and fat contents during the cooking process would be beneficial to the industry. It would allow the processor to meet the legal requirements for moisture content while maintaining product quality. This study examined methods of analysing MR decay curves as a means of determining moisture contents of a cheese product called cheese analogue.

The T_2 time constant is an indicator of the molecular mobility of water, which is affected by the interactions between water and macromolecules that perturb the motion of water. According to one theory, as moisture content increases multilayers of water are formed, with each layer having successively less interaction with the macromolecules (Ruan & Chen, 1998). Water molecules are found in a variety of different environments giving rise to a spectrum of spin-spin relaxation times. Therefore, the measured relaxation decay is a sum of contributions from spins that may have sampled many different environments. The complexity of food systems makes it appropriate to expect them to have multiple relaxation times.

Multi-exponential analysis has been used to interpret the T_2 relaxation data of a variety of dairy products (Brosio *et al.* 1983; Schmidt, 1990; Brosio & Barbieri, 1996; Chaland *et al.* 2000; Curtis *et al.* 2000). The major ingredients of cheese are water, casein, and milk fat. The relaxation of water bound to casein is faster than the relaxation of milk fat or vegetable oil. Therefore, the overall relaxation rate of cheese should include relatively fast and relatively slow components. The one common approach to analysing this type of decay curve is to fit it to a model consisting of the sum of several exponential components. For example, Chaland *et al.* (2000) used a two-term exponential model to describe the low field (20 MHz) T_2 relaxation curves of the liquid phase of cheeses.

When there are more than two components, it is very difficult to deconvolute the decay curve (i.e. to estimate individual values of the relaxation time constants). This difficulty has been addressed in the literature (Kroeker & Henkelman, 1986; Whittall & Mackay, 1989; Clayden & Hesler, 1992). Therefore, other models representing the relaxation decay should be investigated.

An alternative approach to modelling the relaxation is to categorize the relaxation of cheese as non-exponential. This type of relaxation can occur when there is a collection of molecular environments each relaxing in an exponential fashion at a characteristic rate (Lindsey & Patterson, 1980). Such distributions of relaxation times have been modelled using a Kohlrausch-Williams-Watts stretched exponential model. The model has been used primarily for porous rock systems and polymers (Kenyon *et al.* 1986; Howard *et al.* 1993; Narayanan *et al.* 1995; Choi *et al.* 1998; Laviolette *et al.* 1999). As mentioned previously, there are probably at least several molecular environments in cheese. This makes cheese relaxation behaviour a suitable candidate for the stretched exponential model. The single-term model has the advantage of having fewer parameters to fit compared with the more commonly used multi-exponential models.

The sensory properties of cheese and processed cheese are greatly affected by variations in composition, such as changes in moisture and fat contents, or a change in the type of fat (Chen *et al.* 1975; Marshall, 1990; Lobato-Calleros *et al.* 1998). Variation in composition is one factor that makes it difficult to study the effect of composition on the relaxation times of real cheese. Therefore, cheese analogues were used for the experiments described in this paper. Cheese analogues have a texture similar to real cheese and are made by combining water, casein, and fat without aging (Marshall & Kirby, 1988). They allow better control over composition and eliminate variations caused by aging. In these experiments, milk fat and vegetable oil were used as two different sources of fat. Vegetable oil was used in the original recipe for making cheese analogue that was obtained from NZMP (New Zealand Milk Products, 1999) and vegetable oil is an ingredient of some commercial cheese sauces. Both types of fat have been used in previous studies of cheese analogue (Lobato-Calleros *et al*. 1998). However, vegetable oil has a longer T_2 than milk fat, permitting a greater separation in T_2 values of the fat and water components. Type of fat influences cheese properties because the two fats have different fatty acid profiles and melting points (Walstra & Jenness, 1984).

The objectives of this study were:

1. To investigate the use of the multi-exponential and stretched exponential models for analysis of T_2 relaxation curves and to determine how the models are affected by the moisture and fat contents of the cheese analogue.

2. To compare the stretched exponential model to the multi-exponential model on the basis of complexity, the insights provided into relaxation behaviour, and efficacy in predicting moisture content.

MATERIALS AND METHODS

Sample preparation

Cheese analogues were prepared using the ingredients and proportions given in Table 1 (NZMP, 1999). The majority (850 g/kg) of the distilled water was placed in a beaker. After addition of disodium phosphate, sodium citrate, sodium chloride, and potassium sorbate, the solution was heated to 90 °C. Edible rennet casein, ALAREN 771, 819 g/kg protein and 118 g/kg moisture (New Zealand Milk Products, Inc., Santa Rosa, CA, USA) was added to the heated solution, and allowed to hydrate for 15 min. Citric acid was dissolved in the remaining distilled water, which was at room temperature, while the fat was heated to 90 °C. The hydrated casein and sodium aluminium phosphate were mixed in the mixing bowl of a jacketed Hobart Mixer (Model C-100, Hobart Corporation, OH, USA) maintained at 90 °C and mixed at a low speed until a homogenous mass was formed. The heated fat and the citric acid solution were added and the mixture was mixed continuously until it was homogenous. Total mixing time was about 8 min.

Immediately after mixing, hot samples were placed in two 25-ml clear glass vials, which were subsequently cooled at 5 °C for 24 h. Then, the sample was warmed to room temperature (22·5 °C) over a period of 24 h and the MR experiments were performed in an air-conditioned lab. In each sample, the proportions of fat and non-fat solids in dry matter were held constant. Moisture contents ranged from 424·5 g/kg, which is typical of processed cheese, to 501·3 g/kg, which is more typical of processed cheese spread.

Magnetic resonance measurements

Magnetic resonance measurements were made with a D-1000 low field (1150 Gauss, 5·35 MHz) magnetic resonance sensor (Magnetic Instrumentation Inc., Indianapolis, IN, USA). After the two glass vials containing one of the two types of cheese analogue were equilibrated to room temperature (22·5 °C), they were each

Table 1. *Recipe used to prepare cheese analogue samples*

Components	Composition g/kg dry basis
Rennet casein	501·0
Milk fat/vegetable oil	419·0
Sodium chloride	37·3
Citric acid, anhydrous	15·2
Disodium phosphate, anhydrous	14·1
Sodium aluminium phosphate	9·5
Sodium citrate	1·9
Potassium sorbate	1·9

placed in the magnetic resonance sensor sample tube in succession. Their spin-spin relaxation time (T_2) was measured using a Carr-Purcell-Meiboom-Gill (CPMG) sequence. This sequence is described as:

$$90_x - (\tau - 180_y - \tau - \text{echo})_n,$$

where $\tau = 1$ ms. The length of the 90° pulse was approximately 11 μs with a signal to noise ratio of approximately 60. For vials at room temperature, 100 points were acquired while 800 points were acquired for samples heated at 70 °C (see below). Two separate relaxation measurements were made on each vial and analysed independently. Therefore, there was only one scan per measurement.

After completion of measurements at room temperature, the samples were reheated in a forced convection oven for 1 h at one of the specified oven temperatures. Oven temperatures varied from 30 °C to 70 °C in 10 deg C increments. During preliminary experiments, temperatures of several of the samples at each specified temperature were measured before and after the T_2 tests were performed. Even after equilibration for 1 h, initial temperatures measured by inserting a digital thermometer (Model HH-25 KC, Omega Engineering, Inc., Stamford, CT, USA) were 1–5 °C below oven temperature. The higher the oven temperature, the greater the difference between the sample and target (oven) temperature. The preliminary tests indicated that the sample temperature dropped by less than 2 °C during the CPMG-T_2 tests.

After the 1-h equilibration, each sample was inserted into the magnetic resonance sensor sample tube and a spin-spin relaxation experiment was conducted. The total time required to run the MR experiments was approximately 2 min. Immediately after each MR measurement was completed, the sample temperature was determined with the digital thermometer. As a result of the failure to attain oven temperature and the slight cooling (< 2 °C) of the sample during the experiment, the actual temperatures of samples during the test were lower than the target temperature. The temperature reported was the final temperature immediately after the two MR measurements. Based on the results of the temperature measurements during the preliminary test, the short duration of the measurement, and the good agreement between the two successive measurements on a given vial, the actual sample temperature at the time of the measurements should have been very close to the values reported.

Analysis of spin-spin relaxation data

Before fitting the data using Curve Expert® 1.34 (Hyams, 1993), the data were normalized by dividing each echo amplitude by the amplitude of the echo with the greatest amplitude (the initial echo). This eliminated fluctuations caused by variations in sample size.

Two types of models were used to analyse the T_2 signals. The first type was multi-exponential, consisting of two terms when modelling the decay curves of pure fats and three terms when modelling the decay curves of cheese analogues. The second was a stretched exponential model with either one term (pure fats) or two terms (cheese analogues).

Two-term exponential model. Chaland *et al.* (2000) and Budiman *et al.* (2001) reported that the relaxation decay curves of milk fats are best described by a two-term exponential model. Budiman (2001) also reported that vegetable oil was best described by a two-term model. Therefore, curves from both fats were fitted to the following two-term exponential model with relaxation constants T_{2b} and T_{2c}:

$$\text{normalized signal} = \beta \exp\left(\frac{-t}{T_{2b}}\right) + \chi \exp\left(\frac{-t}{T_{2c}}\right), \tag{1}$$

where β and χ are coefficients giving the relative contribution of each term. When the model shown in eqn 1 was used for pure milk fat and pure vegetable oil, the relaxation time constants were designated T_{2b} and T_{2c}. Using 'b' and 'c' rather than 'a' and 'b' reduces confusion when, as described below, these relaxation time constants are incorporated into the three-term exponential model of cheese analogue. The values of β, T_{2b}, χ, and T_{2c} were determined by fitting the data using Curve Expert® 1.34. The coefficients β and χ indicate the relative contributions of the T_{2b} and T_{2c} terms to the signal.

Three-term exponential model. The two major components contributing to the MR signal of cheese are water and fat. As stated previously, relaxation decay curves from CPMG tests of pure fats are best described by a two-term exponential. Therefore, it seems appropriate to add an additional exponential term and use a three-term exponential model to fit the cheese analogue decay curves. This allows two terms for the fat component and one term for water. The result is the following three-term exponential model with relaxation constants T_{2a}, T_{2b}, and T_{2c}:

$$\text{normalized signal} = \alpha' \exp\left(\frac{-t}{T_{2a}}\right) + \beta' \exp\left(\frac{-t}{T_{2b}}\right) + \chi' \exp\left(\frac{-t}{T_{2c}}\right), \tag{2}$$

where α', β', and χ' are coefficients giving the relative contribution of each term. After the signal was normalized, the T_{2b} and T_{2c} values were obtained from a two-term exponential fit of samples of either pure milk fat or pure vegetable oil. To differentiate the coefficients obtained from pure fats from those obtained from cheese analogue, an apostrophe sign was used to designate coefficients obtained from cheese analogue. The value T_{2a} and coefficients α', β', and χ' were determined by fitting the data to the model using Curve Expert® 1.34. Just as in the case of the two-term model, the coefficients α', β', and χ' indicate how much each relaxation term contributes to the signal. The ratio of coefficients β' and χ' in the cheese analogue was fixed so that it was the same as the ratio of coefficients β and χ in either pure milk fat or pure vegetable oil. Fixing T_{2b} and T_{2c} and the ratio of β' to χ' is based on the assumption that the T_2 decay of fats is not affected by their incorporation into the cheese.

Single-term stretched exponential model. Since the two-term exponential analysis of the relaxation signal of pure fats revealed two different relaxation times, their T_2 decay curves were also fitted to a stretched exponential model. This model is very convenient because it is compact, consisting of only two parameters ($T_{2\beta}$ and n). It is described as follows:

$$\text{normalized signal} = \exp\left[-\left(\frac{t}{T_{2\beta}}\right)^{n}\right], \tag{3}$$

where $T_{2\beta}$ is the relaxation constant and n is the stretched exponential parameter. In this type of model the parameter n usually ranges from slightly greater than 0 to 1. The case of $n \rightarrow 0$ has been justified theoretically as describing an infinitely broad distribution. When $n = 1$ the model describes a homogenous system with a single relaxation time (Williams & Watts, 1970). In this paper, the average relaxation time constant for the stretched exponential model is denoted using a Greek letter to distinguish it from the multi-term exponential time constant. The relaxation time constants of samples of pure fat were denoted as $T_{2\beta}$. The symbol 'β' was used instead of 'α' to minimize confusion when these values were used in the two-term stretched exponential model described in the following section.

Two-term stretched exponential model. As mentioned above, analysis of CPMG tests of pure milk fat and vegetable oil revealed that they were best described by a single-term stretched exponential model. Consequently, it was hypothesized that the relaxation-decay curve model of cheese analogues has two different stretched exponential terms, one contributed by moisture and the other by fat. This led to the development of the following model with relaxation constants $T_{2\alpha}$ and $T_{2\beta}$:

$$\text{normalized signal} = \alpha \exp\left[-\left(\frac{t}{T_{2\alpha}}\right)^{n_1}\right] + \beta \exp\left[-\left(\frac{t}{T_{2\beta}}\right)^{n_2}\right]. \tag{4}$$

The coefficients α and β indicate the relative contributions of the $T_{2\alpha}$ and $T_{2\beta}$ components to the signal where $\beta = 1-\alpha$, and the n_i's are the stretched exponential parameters. It seems reasonable that the n_1 parameter of the model will indicate whether the relaxation of the moisture in the cheese analogue, like the relaxation of the fat, has more than one relaxation time.

Moisture and fat content determination

Moisture content was determined on triplicate subsamples obtained from cheese analogue blocks. A vacuum oven (Squaroid Duo-Vac Oven, Lab-Line Instruments, Inc., Melrose Park, IL, USA) was used to dry the samples at 100 °C for 5 h under pressures less than 66 cm Hg (Marshall, 1992; AOAC, 1995). Fat content was determined using the Babcock method (Marshall, 1992).

Statistical analysis

A multiple comparison of means was performed using the Tukey's test available from SAS® statistical software (SAS Institute Inc., Cary, NC, USA). Differences significant at the 5% level were determined.

RESULTS AND DISCUSSION

The moisture and fat contents for the milk fat and vegetable oil cheese analogues are shown in Table 2. The moisture content of the seven milk fat analogue samples varied from 424·5 to 501·3 g/kg, while the moisture of the six vegetable oil analogue samples varied from 432·3 to 498·4 g/kg on a wet basis.

Table 2. *Parameters of two-term exponential models and stretched exponential models of T_2 relaxation of pure milk fat and pure vegetable oil*

	Two-term exponential						Single-term stretched exponential		
Temp.† (°C)	β	T_{2b} (ms)	χ	T_{2c} (ms)	Average T_2 ‡ (ms)	SE	$T_{2\beta}$ (ms)	n	SE
Pure milk fat									
22	0·63	39	0·37	146	78·5	0·0033	66	0·78	0·0105
28	0·60	53	0·40	183	104·3	0·0026	89	0·80	0·0099
36	0·56	72	0·44	233	142·8	0·0024	125	0·82	0·0083
45	0·60	111	0·40	354	208·6	0·0028	182	0·82	0·0086
54	0·44	120	0·56	330	237·7	0·0020	218	0·87	0·0055
63	0·41	167	0·59	450	334·5	0·0023	310	0·87	0·0046
Pure vegetable oil									
22	0·61	52	0·39	226	120·8	0·0033	97	0·74	0·0106
28	0·57	70	0·43	276	159·2	0·0025	133	0·77	0·0101
36	0·56	94	0·44	351	207·5	0·0022	176	0·78	0·0095
45	0·56	127	0·44	471	277·8	0·0027	236	0·78	0·0091
54	0·46	152	0·54	512	346·9	0·0022	307	0·82	0·0066
63	0·46	196	0·54	655	442·6	0·0026	392	0·82	0·0064

† Temperature measured upon completion of the MR test.

‡ Weighted average equal to $\beta\ T_{2b} + \chi\ T_{2c}$.

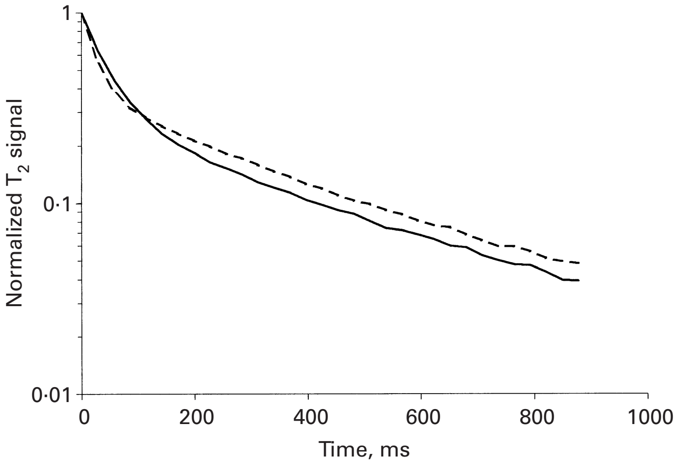

Fig. 1. Semi-log plot of normalized T_2 relaxation curves as a function of time for milk fat cheese analogue samples tested at 63 °C. Samples contained either - - - -, 424·5 or, ——, 501·3 g moisture/kg cheese.

Figure 1 is a semi-log plot of the normalized T_2 decay curves for the 63 °C milk fat cheese analogue samples with the highest and lowest moistures. Note that the shape of the curves varies with moisture. If the decay curve could be described by a single exponential term, then the points should fall on a straight line with slope equal to $1/T_2$. It is obvious that the relationship is not linear and that the T_2 decay curve exhibits a multi-exponential behaviour.

Multi-exponential models

Two-term exponential model. The T_2 curves of pure milk fat and pure vegetable oil were studied in detail because fat is one of the major components of cheese analogue. Furthermore, it appeared that the T_2 of fat was unaffected by incorporation into the cheese. The fat was probably in the form of globules and therefore, when the fat was mixed into the cheese analogue, the other components had relatively little effect on the fat's T_2 relaxation time (Callaghan *et al.* 1983; Chaland *et al.* 2000). Values of T_{2b} and T_{2c} for the two-term pure milk fat and vegetable oil models are summarized in Table 2. Weighted averages of T_{2b} and T_{2c} (equal to $\beta\ T_{2b}+\chi\ T_{2c}$) are included.

Three-term exponential model. As mentioned previously, a three-term exponential model (eqn 2) allows two terms to be used to describe the fat component in the cheese analogue and an additional term to describe the water. There are five unknown parameters in the three-term exponential model and therefore the model-fitting process is numerically ill-conditioned (Clayden & Hesler, 1992). However, this problem can be eliminated by fixing the T_2 values associated with the fat. Callaghan *et al.* (1983) reported that the T_2 values of milk fat in cheese and pure milk fat are similar. In the three-term model, the data were analysed by setting T_{2b} and T_{2c} equal to the values obtained from two-term exponential fits of the spin-spin relaxation curves of pure milk fat or vegetable oil samples.

Table 3 summarizes the values of α', T_{2a}, β', and χ' determined by fitting the T_2 curves of milk fat and vegetable oil cheese analogues to a three-term exponential model (eqn 2). All of the analyses reported in this paper were conducted on the samples at six temperatures (Budiman, 2001). The average temperature during the test ranged from 22 °C to 63 °C. However, only values for the lowest (22 °C) and

Table 3. *Parameters of three-term exponential models of T_2 relaxation of milk fat and vegetable oil cheese analogues. Values of T_{2b} and T_{2c} at a given temperature were obtained from the T_2 values of pure milk fat and vegetable oil listed in Table 2*

Moisture content, g/kg	α'	COV‡ (%)	T_{2a} (ms)	COV‡ (%)	β'	COV‡ (ms)	χ'	COV‡ (%)	SE
Milk fat, 22 °C†									
424·5	0·70	1·2	15·1[a]	0·3	0·19	2·8	0·11	2·8	0·0042
438·7	0·68	0·5	15·4[a]	0·7	0·20	1·0	0·12	1·0	0·0039
451·6	0·80	0·3	17·2[b]	1·0	0·13	1·2	0·07	1·2	0·0044
472·0	0·76	0·8	19·0[c]	1·3	0·15	2·5	0·09	2·5	0·0038
482·0	0·76	0·2	19·6[c]	0·3	0·15	0·8	0·09	0·8	0·0035
495·9	0·76	0·2	21·8[d]	1·2	0·15	0·8	0·09	0·8	0·0031
501·3	0·80	0·6	22·6[d]	1·6	0·13	2·4	0·07	2·4	0·0037
Milk fat, 63 °C†									
424·5	0·58	1·0	22·6[a]	0·6	0·17	1·5	0·25	1·4	0·0073
438·7	0·61	0·7	24·9[a]	0·9	0·16	1·1	0·23	1·1	0·0055
451·6	0·62	0·8	27·8[b]	1·2	0·16	1·3	0·22	1·2	0·0045
472·0	0·63	0·9	32·2[c]	1·9	0·15	1·6	0·22	1·6	0·0055
482·0	0·64	1·0	33·9[c]	0·7	0·15	1·8	0·21	1·7	0·0044
495·9	0·64	0·7	35·3[d]	1·3	0·15	1·2	0·21	1·2	0·0058
501·3	0·65	1·1	39·7[e]	0·6	0·14	2·0	0·21	2·0	0·0056
Vegetable oil, 22 °C†									
432·3	0·59	0·2	14·2[a]	0·7	0·25	0·4	0·16	0·4	0·0028
436·4	0·60	0·5	14·9[a]	1·8	0·24	0·8	0·15	0·8	0·0032
459·6	0·66	0·6	17·3[b]	1·7	0·21	1·2	0·13	1·2	0·0036
480·4	0·63	0·4	18·7[c]	1·0	0·22	0·8	0·14	0·8	0·0025
484·2	0·67	0·3	19·2[c]	1·9	0·20	0·7	0·13	0·7	0·0028
498·4	0·68	0·7	22·3[d]	2·0	0·20	1·4	0·12	1·4	0·0028
Vegetable oil, 63 °C†									
432·3	0·63	0·6	25·1[a]	1·1	0·17	1·0	0·20	1·0	0·0024
436·4	0·61	0·8	24·8[b]	1·9	0·18	1·2	0·21	1·2	0·0044
459·6	0·65	0·6	29·8[c]	1·6	0·16	1·1	0·19	1·1	0·0033
480·4	0·66	0·5	33·6[d]	1·2	0·16	1·0	0·18	1·0	0·0029
484·2	0·66	0·4	35·1[e]	0·9	0·16	0·8	0·18	0·8	0·0035
498·4	0·66	0·2	38·2[f]	1·3	0·16	0·4	0·19	0·4	0·0037

† Temperature measured upon completion of the MR test.

‡ COV, Coefficient of Variation for two tests on each of two bottles.

[a,b,c,d,e,f] Means with the same letter within each temperature are not significantly different. $P<0{\cdot}05$. DF = 3.

highest (63 °C) temperatures are reported in this paper. In all cases, results for the intermediate temperatures were similar. The coefficient α' was greater than the sum of coefficients β' and χ' for all samples. This is consistent with the assumption that α' was associated with the water in the cheese. Although the trend was for α' to increase with an increase in moisture content while $\beta'+\chi'$ decreased, the change was not smooth.

At a given temperature, linear regression analysis of the combined data set (both milk fat and vegetable oil cheese analogues) showed that there was a linear relationship between moisture content and T_{2a} values (Fig. 2). The coefficients of correlation were 0·946 and 0·980 at 22 °C and 63 °C, respectively.

Stretched exponential model

Single-term stretched exponential model. As shown in Table 2, the relaxation decay curves of pure milk fat and vegetable oil fit a two-term exponential model. This means that pure milk fat and vegetable oil can be modelled using a single-term stretched exponential model with $n < 1$. The results for pure milk fat and vegetable oil at 22 °C and 63 °C are summarized in Table 2. The standard errors of fitting the

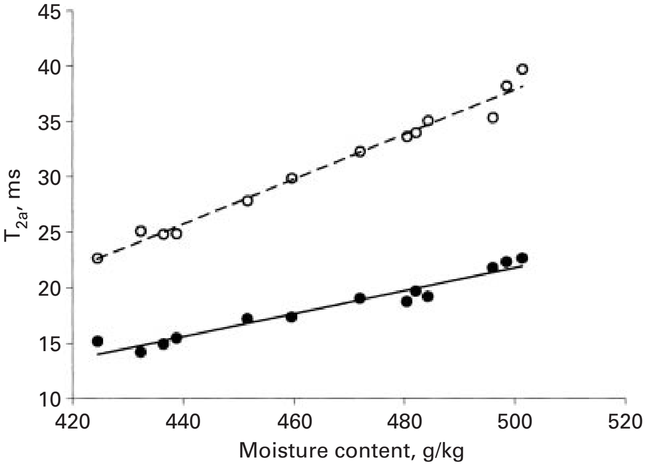

Fig. 2. T_{2a} values from the three-term exponential model versus moisture content of cheese analogue samples made with milk fat and vegetable oil tested at ●, 22 °C ($r^2 = 0{\cdot}946$) and ○, 63 °C ($r^2 = 0{\cdot}980$).

pure milk fat and vegetable oil to the single-term stretched exponential were approximately three times greater than the standard errors for fitting them to the two-term exponential model (Table 2).

Two-term stretched exponential model. When modelling the T_2 decay curve of cheese analogue, the same approach used for the multi-exponential model was used for the stretched exponential model. An additional term was added to account for the water. The first term ($T_{2\alpha}$) of the stretched exponential model describes the water and the second term ($T_{2\beta}$) describes the fat. It is possible that the water in the cheese sample, which is bound in the protein matrix, would have more than one T_2 value.

The data were analysed by fixing $T_{2\beta}$ in eqn 4 as the $T_{2\beta}$ value from a single-term stretched exponential model of either pure milk fat or pure vegetable oil samples (Table 2). Table 4 summarizes the results of fitting the model to the T_2 data from the milk fat and vegetable oil cheese analogues at 22 °C and 63 °C. The $T_{2\alpha}$ values for both samples increased as the moisture content increased. Most of the differences associated with moisture were statistically significant ($P < 0{\cdot}05$). The COV's were less than or equal to 2·7%. The coefficient α, which describes the water component, was greater than the coefficient β and it tended to increase as the proportion of moisture increased. However, the increase in α with an increase in moisture was not smooth. The coefficient β tended to decrease as the proportion of fat decreased.

When the $T_{2\alpha}$ values from milk fat and vegetable oil cheese analogues were combined into a single data set (13 values at a given temperature), linear regression analysis showed that there was a linear relationship between $T_{2\alpha}$ and moisture content for both analogues. The $T_{2\alpha}$ values increased linearly as the moisture content increased (Fig. 3). The values of r^2 were 0·950 and 0·966 at 22 °C and 63 °C, respectively.

For both cheese analogues the values of n_1 were close to one and they decreased only slightly as the temperature increased to 45 °C. As mentioned previously, when $n = 1$ the model describes a homogeneous system with a single relaxation time. Therefore, the results suggest that the T_2 decay of the water in the cheese can be adequately described by a single exponential term.

For both cheese analogue samples there is no definite trend in n_2 at a given temperature. They were similar in magnitude to the n values obtained from a single-

Table 4. *Parameters of two-term stretched exponential models of T_2 relaxation of milk fat and vegetable oil cheese analogues. Values of $T_{2\beta}$ at a given temperature were obtained from the T_2 values of pure milk fat and vegetable oil listed in Table 2*

Source of fat	Temp.† (°C)	Moisture content (g/kg)	α	COV‡ (%)	$T_{2\alpha}$ (ms)	COV‡ (%)	n_1	COV‡ (%)	β	COV‡ (%)	n_2	COV‡ (%)	SE
Milk fat	22	424·5	0·68	1·2	15·8^{a}	1·8	1·00	1·9	0·32	2·6	0·77	2·3	0·0179
		438·7	0·66	0·5	16·4^{a}	0·3	1·01	0·8	0·34	0·9	0·75	0·9	0·0065
		451·6	0·78	0·6	17·2^{b}	0·4	1·00	1·1	0·22	2·1	0·82	2·0	0·0164
		472·0	0·76	2·1	20·0^{c}	2·1	1·00	1·2	0·24	6·7	0·74	3·6	0·0266
		482·0	0·75	1·1	20·4^{c}	1·4	1·02	0·6	0·25	3·3	0·75	2·1	0·0160
		495·9	0·75	1·1	22·6^{d}	0·7	1·02	0·9	0·25	3·2	0·74	2·2	0·0166
		501·3	0·78	0·4	22·8^{d}	1·8	1·01	0·6	0·22	1·3	0·79	2·1	0·0163
	63	424·5	0·55	1·2	24·2^{a}	1·2	0·98	0·4	0·45	1·1	0·74	0·9	0·0068
		438·7	0·58	0·8	26·0^{b}	1·0	0·97	0·7	0·42	1·1	0·76	1·0	0·0074
		451·6	0·59	0·9	28·9^{c}	1·3	0·97	1·4	0·41	1·3	0·77	1·1	0·0086
		472·0	0·61	1·0	34·9^{d}	2·7	0·96	0·6	0·39	1·5	0·75	1·4	0·0108
		482·0	0·62	1·1	35·8^{d}	1·1	0·96	0·6	0·38	1·8	0·77	1·2	0·0090
		495·9	0·62	0·7	38·7^{e}	1·1	0·95	0·4	0·38	1·2	0·74	0·6	0·0043
		501·3	0·63	1·3	43·9^{f}	1·3	0·96	1·5	0·37	2·2	0·73	1·2	0·0087
Vegetable oil	22	432·3	0·58	0·4	16·5^{a}	1·0	1·02	0·6	0·42	0·6	0·68	1·3	0·0032
		436·4	0·59	0·3	16·7^{a}	0·9	1·01	1·2	0·41	0·5	0·71	1·1	0·0031
		459·6	0·64	0·6	18·8^{b}	1·7	1·01	0·4	0·36	1·1	0·72	0·6	0·0033
		480·4	0·64	0·2	21·8^{c}	1·7	1·01	0·6	0·36	0·3	0·67	1·0	0·0028
		484·2	0·66	0·5	21·6^{c}	1·4	1·01	1·4	0·34	1·0	0·68	0·5	0·0030
		498·4	0·67	0·2	24·4^{d}	0·8	1·02	0·9	0·33	0·3	0·70	1·4	0·0028
	63	432·3	0·61	0·7	25·6^{a}	1·1	0·95	0·8	0·39	1·0	0·78	0·6	0·0024
		436·4	0·59	0·9	26·2^{a}	1·2	0·95	0·6	0·41	1·3	0·74	1·2	0·0028
		459·6	0·63	0·7	30·1^{b}	1·7	0·95	0·8	0·37	1·2	0·81	0·5	0·0026
		480·4	0·64	0·6	34·7^{c}	1·1	0·93	0·7	0·36	1·1	0·78	0·7	0·0023
		484·2	0·65	0·5	36·4^{d}	0·8	0·95	1·3	0·35	1·0	0·77	0·4	0·0031
		498·4	0·64	0·2	40·8^{e}	1·3	0·94	0·7	0·36	0·4	0·74	0·8	0·0025

† Temperature measured upon completion of the MR test.

‡ COV = Coefficient of Variation for two tests on each of two bottles.

^{a,b,c,d,e,f} Means with the same letter within each temperature are not significantly different. $P < 005$. DF = 3.

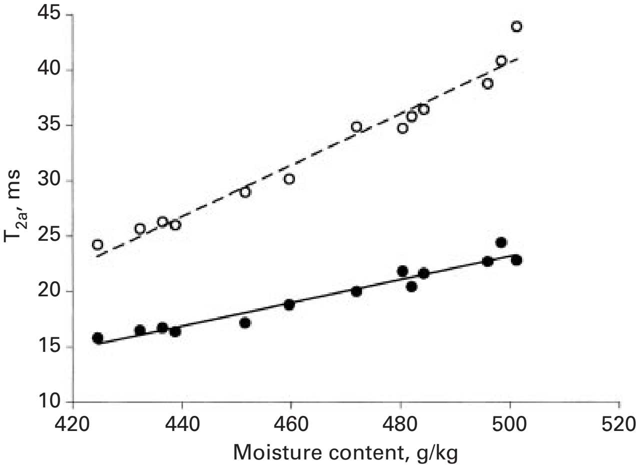

Fig. 3. $T_{2\alpha}$ values from the two-term stretched exponential model versus moisture content of cheese analogue samples made with milk fat and vegetable oil tested at ●, 22 °C ($r^2 = 0{\cdot}950$) and ○, 63 °C ($r^2 = 0{\cdot}966$).

term exponential model of pure milk fat and vegetable oil (Table 3). This may mean that the distribution of T_2 values changes only slightly as the proportion of fat changes.

Comparison of three-term exponential and two-term stretched exponential models

The two-term stretched exponential model is an alternative for analysing the relaxation time constants of complex food systems such as analogue cheese. In this study, it was compared to the three-term exponential model, which was found to be the most appropriate multi-exponential model for cheese analogue. Both methods had six unknown parameters. When applied to normalized T_2 decay curves, only five parameters are independent (α', β', and χ' sum to one in the three-term exponential while α and β sum to one in the stretched exponential model). The number of parameters to be fitted was reduced further by fixing the T_2 values of those terms in each model that described the contribution of fat, and by fixing the ratio of β' to χ' in the three-term exponential model. This left three parameters to be fitted in both of the models.

One of the primary criteria for comparison is efficacy in predicting the moisture content of the cheese analogues. The values of r^2 for a linear relationship between moisture content and either T_{2a} (three-term exponential) or $T_{2\alpha}$ (stretched exponential) were nearly equal. Furthermore, the standard errors for fitting of the models to the decay curves were almost identical for vegetable oil cheese analogue. However, for milk fat cheese analogue the standard errors for fitting of the two-term stretched exponential model were greater than those of the three-term exponential model (Tables 3 and 4).

One advantage of using the two-term stretched exponential model is that it is relatively easy to adjust the relaxation time constant, $T_{2\beta}$ in eqn 4, for changes in product temperature. Values of $T_{2\beta}$ for a single-term stretched exponential model (eqn 3) can be determined for pure milk fat and vegetable oil at various temperatures and an equation can be developed that relates $T_{2\beta}$ to temperature. For the three-term exponential model, equations must be developed for both T_{2b} and T_{2c} as a function of temperature. However, variations in fat composition could require frequent adjustments. Moreover, as shown in Table 2, temperature affected not only the T_2 values but also the coefficients. This would make it more difficult to take into account product temperature fluctuations when using the three-term exponential model for predicting moisture content.

On the other hand, there are disadvantages of using the stretched exponential model. The greater standard errors for the two-term stretched exponential model of milk fat cheese analogue have already been mentioned. Another disadvantage of using the two-term stretched exponential model is that it is more difficult to interpret the meaning of changes in parameters, especially the parameter n. Interpretation of the parameters of the three-term exponential models is straightforward. The value of T_{2a} is related to moisture content while the coefficients of the exponential terms are related to the water and fat contents.

Previous studies of the stretched exponential model were conducted on polymer and porous materials. This study demonstrated that the model can also be applied to complex food systems. However, in order to adequately describe the behaviour of the cheese analogues, a modified stretched exponential model containing two terms was developed. The stretched exponential model was used to analyse T_2 relaxation curves of cheese analogues. It was compared with the more commonly used three-term exponential model. Both models could take into account the contributions of water and fat to the T_2 relaxation curve. Linear regression was used to compare either the $T_{2\alpha}$ values obtained from the stretched exponential model or the T_{2a} values obtained from the three-term exponential model to the moisture contents of cheese analogues.

Both models appeared to adequately describe the effects of variations in moisture content on the T_2 decay curves of cheese analogue. The values of r^2 for the linear regression of the two models were similar and were greater than or equal to 0·946. Although the stretched exponential model offered the advantage of simplicity, for milk fat cheese analogues, the model's standard errors were greater than the standard errors of the three-term exponential model. For vegetable oil cheese analogues, the standard errors of the two models were nearly equal. The observation that $n \cong 1$ in the first term of the two-term stretched exponential model suggests that a single exponential term can be used to describe the T_2 relaxation of the water in the cheese analogues. Additional research is needed to establish the effectiveness of each model when components other than water are varied. Relaxation curves can be influenced by spacing between pulses (2τ), which was 2 ms in this study (Hills *et al*. 1990). Therefore, an investigation of the effect of varying τ time should be conducted to determine how it affects the modelling of T_2 relaxation.

This research was funded by a grant from Dairy Management Inc. Technical support and MR equipment were provided by Magnetic Instrumentation, Inc. of Indianapolis, Indiana. In addition, the rennet casein (ALAREN 771) was provided by New Zealand Milk Products, Inc. The authors gratefully acknowledge these sources of support.

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