Evaluation of an improved tracer method to monitor cheese curd syneresis at varying milk fat levels in a cheese vat

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Previous studies have reported that cheese curd syneresis kinetics can be monitored by dilution of chemical tracers, such as Blue Dextran, in whey. The objective of this study was to evaluate an improved tracer method to monitor whey volumes expelled over time during syneresis. Two experiments with different ranges of milk fat (0-5% and 2.3-3.5%) were carried out in an 11 L double-O laboratory scale cheese vat. Tracer was added to the curd-whey mixture during the cutting phase of cheese making and samples were taken at 10 min intervals up to 75 min after cutting. The volume of whey expelled was measured gravimetrically and the dilution of tracer in the whey was measured by absorbance at 620 nm. The volumes of whey expelled were significantly reduced at higher milk fat levels. Whey yield was predicted with a SEP ranging from 3.2 to 6.3 g whey/100 mL of milk and a CV ranging from 2.03 to 2.7% at different milk fat levels.

Bewertung einer verbesserten Tracer-Methodik zur Überprüfung der Synerese von Käsebruch bei unterschiedlichen Milchfettgehalten in einer Käsewanne

Frühere Studien haben gezeigt, dass die Kinetik der Synerese von Käsebruch durch Verdünnung eines chemischen Tracers, zum Beispiel Dextranblau, in der Käsemolke überprüft werden kann. Die Zielsetzung dieser Studie war es, eine verbesserte Tracer-Methodik zu bewerten, um das Molkenvolumen, das während der Synerese freigesetzt wird, zu ermitteln. Zwei Versuche mit Milchen unterschiedlicher Fettgehalte (0-5 und 2,3-3,5 %) wurden in 11 Liter-Käsewannen durchgeführt. Der Tracer wurde der Käsebruch-Molkenmischung während der Schneidephase des Käsebruchs zugesetzt, und die Proben wurden in Intervallen von 10 bis 75 min nach dem Schneiden entnommen. Das Volumen der ausgepresster Molke wurden gravimetrisch gemessen, und die Verdünnung des Tracers in der Molke wurde durch die Absorbanz bei 620 nm bestimmt. Die Volumina der freigesetzten Molke waren bei höheren Milchfettgehalten deutlich reduziert. Die Molkenmenge wurde mit einem SEP zwischen 3,2 und 6,3 g Molke/100 ml Milch und einem CV zwischen 2,03 und 2,7% bei den unterschiedlichen Milchfettgehalten vorausgesagt.

51 Syneresis (tracer method) **51 Synerese** (Tracer-Methode)

1. Introduction

Syneresis is the usual concentration mechanism in making cheese from renneted or acidified milk. According to WALSTRA *et al.* (1), the methods employed to measure syneresis as a function of time can be classified in four groups, namely, (a) measurement of the shrinkage of curd, (b) estimation of curd moisture content (or dry matter), (c) determination of curd density, and (d) determination of the amount of whey expelled through the addition of a tracer to the curd-whey mixture. Blue Dextran 2000 has been used to monitor syneresis on several occasions (2, 3, 4, 5, 6). CASTILLO *et al.* (7, 8) and FAGAN *et al.* (9, 10) monitored the amount of whey expelled during curd shrinkage using novel optical sensors. EVERARD *et al.* (11) showed that computer vision technology could be used to monitor syneresis.

The principle of the tracer method is as follows: a chemical agent added to the mixture of whey and curd becomes logarithmically diluted in the whey as further whey is expelled and its diminishing concentration indicates the rate of syneresis. The chemical agent added to the whey must not be toxic, should not disturb the process of syneresis, nor be adsorbed at the surface of the curd or diffuse into it (3).

ZVIEDRANS and GRAHAM (6) claimed that the main advantage of a tracer method is the measurement of whey volume at the early stages of syneresis. The main disadvantage is that a certain quantity of tracer associates with the curd causing syneresis to be overestimated.

The tracer method followed in these experiments was adapted from the method developed by ZVIEDRANS and GRAHAM (6). The method was also employed by VON BÜELER (5) and GRUNDELIUS (2). However, neither of these studies used a procedure to clarify whey samples from a cheese vat as syneresis was induced by suspending single curd particles in very small volumes of ultra filtrated milk permeate, and therefore fat and protein molecules did not interfere with the absorbance measurements carried out.

Previous studies have not investigated the use of a tracer method to monitor syneresis at a range of syneresis rates or at varying milk composition. Our study deals with the limitations of using the tracer method in a laboratory scale cheese process, where a tracer is suspended in a stirred curd system, to monitor syneresis under simulated commercial cheesemaking conditions. Previously published syneresis results obtained by different laboratory methods are difficult to compare as the conditions under which syneresis was measured, e.g. curd firmness, rate of syneresis, rate of stirring, temperature, pH, etc., were not standardized.

The aim of this study was to evaluate an improved tracer method to monitor cheese curd syneresis at varying milk fat levels in an 11 L double-O laboratory scale cheese vat under simulated commercial cheesemaking conditions.

2. Materials and methods

2.1 Experimental design

Two experiments were carried out in triplicate with different ranges of milk fat content. Experiments A and B consisted, respectively, of three levels of milk fat (0%, 2.5% and 5% fat) and two levels of milk fat (2.3% and 3.5%).

2.2 Preparation of Blue Dextran solution

Preliminary trials indicated that a 2% *w/v* Blue Dextran (BD) solution produced absorbance measurements of whey samples detectable at 620 nm. A 2% *w/v* solution of Blue Dextran (Blue Dextran 2000, Sigma Aldrich Ireland Ltd. Dublin, Ireland) was prepared using distilled water. The solution was prepared fresh for each trial.

2.3 Milk preparation and coagulation

Low-heat skim milk powder (SMP) (Irish Dairy Board, Dublin, Ireland) was reconstituted in an 11 L double-O cheese vat (Pierre Guerin Technologies, Mauze, France) using distilled water until a final volume of 11 L. Cream (Dairygold, Cork, Ireland) was also added, to achieve the target fat level, at 42 °C while being stirred at 44 rpm. Fat content of the cream and SMP mixture was determined using a Milkoscan analyser (Milko Scan 605, A/S N. Foss Electric, Denmark). A detailed description of the cheese vat was provided by EVERARD (11).

Calcium chloride was added to the milk (2.04 mM) before it was rapidly cooled to 8 °C under constant stirring at 10 rpm. Milk was held at this temperature overnight. The following day milk was heated to 32 °C and pH was measured and adjusted to 6.5 using HCl (1 M). Compositional analysis of milk was determined at this stage using the Milkoscan analyser.

The rennet used for coagulation was 100% recombinant chymosin (CHY-MAX extra, EC 3.4.23.4, isozyme B, 600 IMCU mL^{-1}; Chr Hansen Ireland Ltd., Ireland). Rennet was added to the milk (0.18 g of chymosin kg^{-1} of milk) in the vat while being stirred constantly at 31 rpm. After 3 min stirring was stopped and the stirrers were replaced with cutting blades.

2.4 Gel cutting, BD addition and whey sampling procedure

Gel cutting time at 32°C was determined using a small amplitude oscillatory rheometer (Bohlin CVO Rheometer, Bohlin, Cirencester, UK) according to EVERARD *et al* (11). When the elastic modulus (G') reached 35 Pa cutting was initiated using the cutting blades. The moment at which cutting started was considered as the reference time (t = 0) for the subsequent steps. The cutting procedure was carried out in three phases as proposed by EVERARD *et al* (12). In the first phase at t = 0 the curd-whey mixture was cut at 10 rpm for 40 s followed by a rest period of 20 s; in the second phase, at t = 1 min, cutting started at 22 rpm for 40 s with a rest period of 20 s; the third phase at t = 2 min was performed as the second one. The cutting blades were then replaced by the stirrers.

In experiment A, 55 mL of the 2% BD solution was carefully poured in the middle of the vat at the second cutting phase. In experiment B, four aliquots of 13.75 mL were uniformly and simultaneously injected into the curd using syringes after the first cutting phase in order to distribute the tracer quicker.

At each sampling time (15, 35, 55, and 75 min after cutting) ~270 mL of curd-whey mixture were sampled into 75 μm sieves and sieve pans (12). The whey and the curd yields were measured at each time point using a precision balance (HC32000 Reflex EE, Avery Weigh-Tronix, Dublin)

2.5 Clarification of whey samples

Following the procedure used by ZVIEDRANS and GRAHAM (6) for clarification of whey samples, a 15 mL whey sample was dispensed into a 50 mL polyethylene centrifuge tube (114 x 28 mm, Sarsted, Nümbrecht, Germany). 1.13 mL of buffer solution (1M K_2HPO_4, pH 6.45, Sigma Aldrich, Seelze, Germany) was added to adjust pH, 0.37mL of detergent 10% (*w/v*) sodium dodecyl sulphate (SDS) (BDH Chemicals Ltd., Poole, UK) was added to precipitate the protein and 15 mL of chloroform (99.8% ACS, Sigma Aldrich, Steinhem, Germany) was added to extract the fat. The mixture was centrifuged (Eppendorf centrifuge 5810R, Eppendorf AG, Hamburg) at 20°C and at 3000 rpm for 15 min.

To obtain a more translucent sample an extra centrifugation step was needed. The supernatant phases were transferred into 1.5 mL Eppendorf tubes (Sarsted, Nümbrecht, Germany) and centrifuged for 10 min at 14000 rpm (Eppendorf centrifuge 5417 C, Eppendorf AG, Hamburg). Supernatants were then transferred to microcuvettes (10 x 4 x 45 mm, Sarsted, Nümbrecht, Germany) and absorbance was measured at 620 nm relative to SMUF (simulated milk ultra filtered) using a Varian Cary 1 UV/Visible spectrophotometer (JVA Analytical, Dublin).

2.6 Spectrophotometric measurements

The predicted whey volume (mL whey/100 mL milk) was calculated by dividing the initial concentration of BD in milk (0.01 g per 100 mL milk) by the previously calculated concentration of BD in each whey sample (g mL^{-1} whey) using Lambert-Beer's Law as follows:

$$A = alc,$$

where A is the absorbance reading (dimensionless) given by the spectrophotometer, α is the absorption coefficient (L g^{-1} cm^{-1}) given by the slope of the calibration curve, l is the path length (cm), and c is the BD concentration (g L^{-1}).

The calibration curve (Fig. 1) was determined with respect to water and SMUF in order to obtain the corresponding BD concentration (g L^{-1}) in the samples at each particular absorbance measurement. SMUF was prepared as described in JENNES and KOOP (13). Preliminary tests were carried out in order to compare the mean absorbance and standard deviation of a number

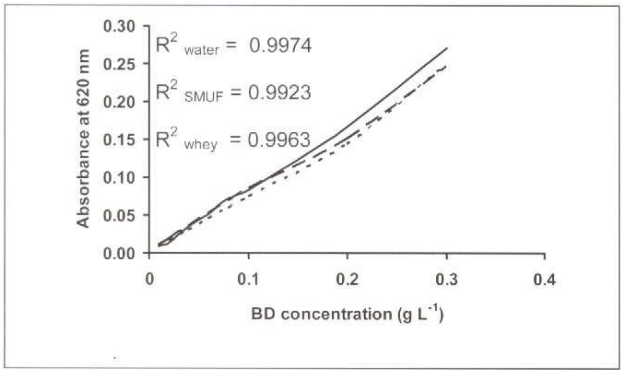

Fig. 1: Calibration curve for BD absorbance in water (—), whey (---) and SMUF (...) solutions.

of 2% BD samples diluted 100 fold with water, SMUF and with whey coming from different milk fat levels. For the samples diluted with whey the analytical procedure was carried out as per the Materials and Methods section. It was shown that the standard curve in SMUF was similar to the standard curve in whey. It was decided to use the standard curve in SMUF as a reference curve as whey composition changed for each treatment and a new standard curve in whey would have been required for each treatment.

2.7 *Statistical analysis*

Repeatability was calculated as the average standard deviation for absorbance, whey volume and predicted whey volume for the three replicates.

Predictability was calculated using SEP for gravimetric and predicted whey volumes.

Analysis of variance was carried out using the general linear model (GLM) procedure in Minitab V15, (Minitab Ltd., Coventry, UK) in order to analyse and quantify (F value) the effect of fat, time and their interaction on the absorbance measurements, gravimetric and predicted whey volumes. Significant differences were determined at $p < 0.05$.

3. Results and discussion

Figure 2 shows the whey volume expelled (mL whey/100 mL milk) in experiment A during 75 min of syneresis, at 10 min intervals, measured gravimetrically and predicted by the tracer method (three replicates). At t = 5 min, the volume of whey predicted was significantly higher than the one measured gravimetrically. This is attributed to incomplete mixing after adding the BD, a factor that seems to be overcome at t = 15 min. Therefore, the 5 min time points were excluded from statistical analysis.

Results showed that the tracer method was able to predict the volume of whey expelled at each time point (15 to 75 min) with a SEP of 6.3 g/100g for 0% fat, 4.7 g/100g for 2.5% fat and 3.2 g/100g for 5% fat. Repeatability of the method was also acceptable, over the range of 0.004 (Table 1).

Predictability at 5 min was improved by injecting the BD solution instead of pouring it, however, these time points were also excluded from the analysis for consistency with experiment A. Repeatability of absorbance readings was not as good, circa 0.01, however, the SEP for prediction of whey volume using milk with 2.3% fat was 7.6 g/100g and for 3.5% fat was 5.9 g/100g which is in the range of predictability determined for experiment A.

Table 1: Repeatability of absorbance (absorbance units), repeatability of predicted whey yield (g/100 mL milk) and predictability (g/100 mL milk) of the tracer method within three replicates at the different milk fat levels.[1]

	Fat level	Repeatability Absorbance (abs. units)	Repeatability Yw_p (g/100 mL milk)	SEP (g/100 mL milk)
Exp A	0%	0.0021	1.26	6.33
	2.50%	0.0048	1.54	4.72
	5%	0.0059	1.39	3.23
Exp B	2.3%	0.014	4.82	7.57
	3.5%	0.011	4.04	5.88

[1] No. of samples = 21, Yw_p = Predicted whey yield from absorbance

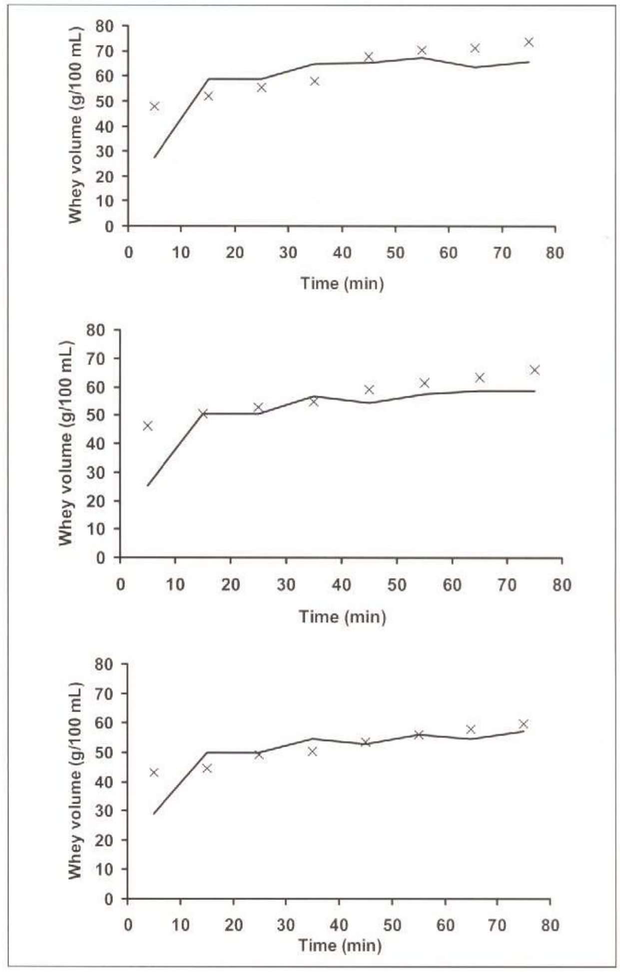

Fig. 2: Predicted (x) and gravimetric (—) whey volumes (g whey/100 mL milk) expelled during syneresis of cheese curd at different milk fat levels: (a) 0% fat in milk, (b) 2.5% fat in milk and (c) 5 % fat in milk. The average of three replicates is represented.

Results showed significant differences in whey volume (measured gravimetrically) for different milk fat levels in both experiments, meaning that it was possible to modify the rate of syneresis by changing the level of fat in the milk (Table 2).

In experiment A, decreasing whey volumes (63.4, 55.3 and 53.6 g/100g) with increasing milk fat levels (0, 2.5 and 5%) could be detected both gravimetrically and

Table 2: Effect of milk fat level on gravimetric whey yield (Yw_g, g/100mL), BD absorbance (Abs) and predicted whey yield from absorbance (Yw_p, g/100mL) at the different fat levels in experiment A and B[1]

Experim.	Fat %	Yw_g	Abs	Yw_p
A	0	63.4[a]	0.112[a]	64.0[a]
	2.5	55.3[b]	0.124[b]	58.2[b]
	5	53.6[c]	0.138[c]	53.0[c]
B	2.3	59.6[a]	0.132[a]	55.7[a]
	3.5	53.2[b]	0.131[a]	55.6[a]

[1]Mean values with same letters are not significantly different ($P<0.05$)

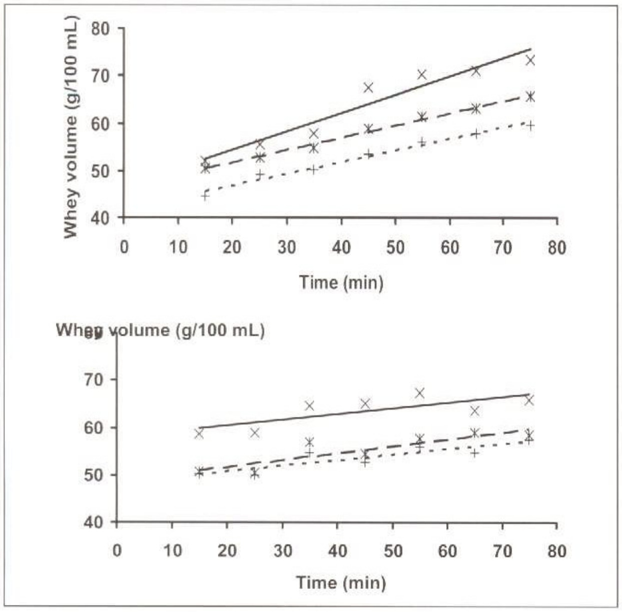

Fig. 3: Linear regression for (a) predicted whey volume and (b) gravimetric whey volumes in Experiment A at the different milk fat levels: 0% (—), 2.5% (---) and 5% (...). The average of 3 replicates is represented

optically (Fig. 3). However, with the decreasing range of fat in experiment B, (2.3 to 3.5%) changes in whey volume could still be detected gravimetrically but not optically (Table 2). This is consistent with the fact that differences in whey volume expelled in this experiment are of a similar range to the repeatability of the method, and hence the errors overlap. Indeed, the difference between the gravimetric whey volumes at the two milk fat levels in experiment B is 6.4 g/100g and the repeatability of the predicted whey volumes ranges from 4 to 4.8 g/100g (Table 1).

Table 3 shows the significance of the effect of experimental variables fat, time and their interaction on the gravimetric whey yield (Yw_g, g/100g), BD absorbance (Abs) and predicted whey yield from absorbance (Yw_p, g/100g). Fat gave the highest F value for the three indices, which implies that fat explains more of the variation than time does. The volumes of whey expelled varied more due to varying milk fat levels than time elapsed during syneresis. Therefore, the errors in prediction are related to the levels of fat used. The interaction between fat and time was only significant in the calculation of the predicted whey volume.

Furthermore, the results obtained for the effect of fat on syneresis rates are in agreement with previous

Table 3: F statistics showing the effect of fat, time and their interactions on gravimetric whey yield (Yw_g, mL whey/100 mL milk), BD absorbance (Abs) and predicted whey yield from absorbance (Yw_p, mL whey/100 mL milk) for the fat levels 0, 2.5 and 5% (63 samples)

	DF	F value[1]		
		Yw_g	Abs	Yw_p
Fat	2	151.4***	108.84***	51.83***
Time	6	23.6***	48.93***	31.15***
Fat*time	12	0.6[ns]	1.56[ns]	7.89***
Error	42	-	-	-
Total	62	-	-	-

[1] F values and significance obtained from mixed model analysis; Significance *** = $P < 0.001$; ns = not significant.

studies which reported significantly reduced syneresis rates at higher milk fat levels (1, 14, 15).

4. Conclusions

The potential of an improved tracer method to monitor cheese curd syneresis at varying milk fat levels in an 11 L double-O laboratory scale cheese vat under simulated commercial cheesemaking conditions was demonstrated. Whey yield was predicted with a SEP ranging from 3.2 to 6.3 g whey/100 mL of milk and a CV ranging from 2.03 to 2.7%.

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