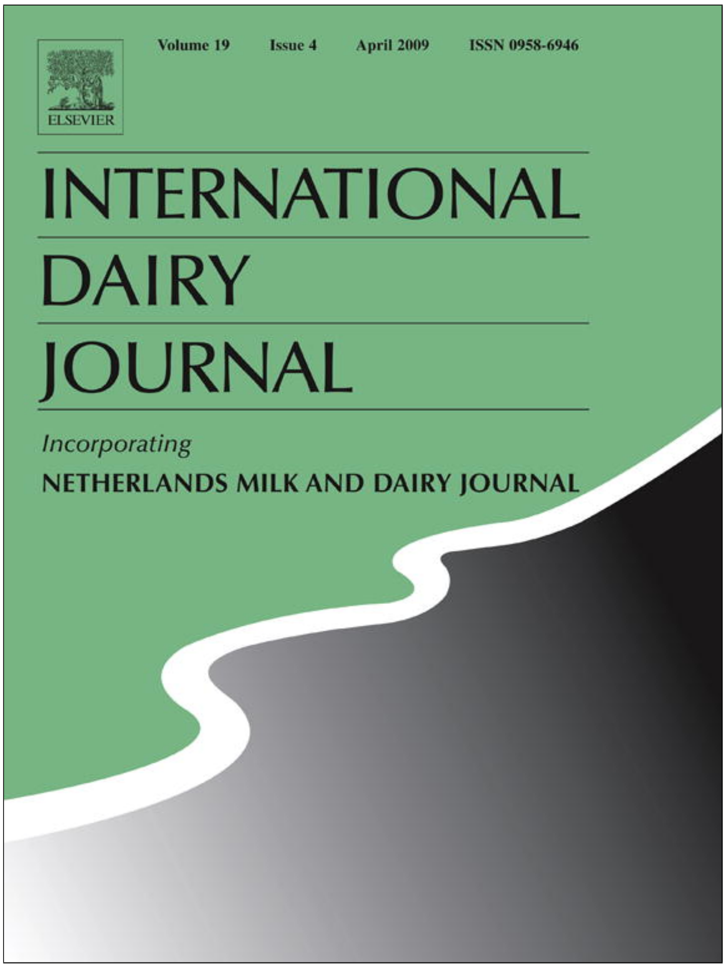
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Effect of milk fat concentration and gel firmness on syneresis during curd stirring in cheese-making

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ABSTRACT

An experiment was undertaken to investigate the effect of milk fat level (0%, 2.5% and 5.0% w/w) and gel firmness level at cutting (5, 35 and 65 Pa) on indices of syneresis, while curd was undergoing stirring. The curd moisture content, yield of whey, fat in whey and casein fines in whey were measured at fixed intervals between 5 and 75 min after cutting the gel. The casein level in milk and clotting conditions was kept constant in all trials. The trials were carried out using recombined whole milk in an 11 L cheese vat. The fat level in milk had a large negative effect on the yield of whey. A clear effect of gel firmness on casein fines was observed. The best overall prediction, in terms of coefficient of determination, was for curd moisture content using milk fat concentration, time after gel cutting and set-to-cut time ($R^2 = 0.95$).

1. Introduction

Daviau et al. (2000) found that the rate and extent of syneresis have an important role in determining some final quality attributes of cheese such as its storage life. Syneresis depends on milk composition, gel firmness at cutting and process conditions, i.e., equipment used, acidification, proteolysis, stickiness of curd grains, etc. (Dejmek and Walstra, 2004; Pearse and Mackinlay, 1989).

A number of factors, including milk composition and pre-treatment, among others, affect the rheological properties of the coagulum at cutting. In general, fat and protein content of milk increase gel firmness (Guinee et al., 1997). It was found that the coagulum strength increased with Ca in milk but a slight decrease was found at high fat levels in milk, i.e., 5–10% (w/w) (Storry, Grandison, Millard, Owen, and Ford 1983). Several other authors demonstrated that an increase of milk fat concentration reduces the syneresis rate (Beeby, 1959; Johnston and Murphy, 1984; Marshall, 1982). Fenelon and Guinee (1999) investigated the effect of milk fat content on rennet coagulation and cheese composition and found that an increase of milk fat concentration resulted in increased milk fat losses in whey.

In a preceding study in our laboratory, it was concluded that casein fines and fat in whey were significantly affected by cutting intensity and stirring speed (Everard et al., 2008). For a better understanding of factors influencing syneresis, it was decided in this study to investigate syneresis over a range of milk fat concentration and firmness levels at gel cutting, which are of direct commercial relevance to cheese manufacture.

The objective of this study was to investigate the influence of varying milk fat concentration in milk at a constant protein level, in combination with cutting the gel at different levels of firmness, on syneresis, while the curd was undergoing stirring. The degree of influence of the experimental factors in predicting indices of syneresis was studied.

2. Material and methods

2.1. Experimental design

A series of trials was carried out in which recombined milk with a range of milk fat levels (FL_m) (0%, 2.5% and 5.0%, w/w), and constant protein level (3.3%, w/w) was coagulated in an 11 L cheese vat (Pierre Guerin Technologies, Mauze, France) and the gel was cut at a range of firmness (G') levels (5, 35 and 65 Pa). Fat level adjustment was determined by least squares optimization of the formulation to target fat and protein levels. This study was undertaken in three replicate blocks ($n = 27$). Rheologically determined gel times ($G' = 0.5$ Pa) were consistent at each milk fat level (SD = 3 min).

2.2. Milk preparation

Whole milk was recombined using medium-heat skim milk powder (5.39 mg g^{-1} of undenatured whey protein nitrogen; Irish

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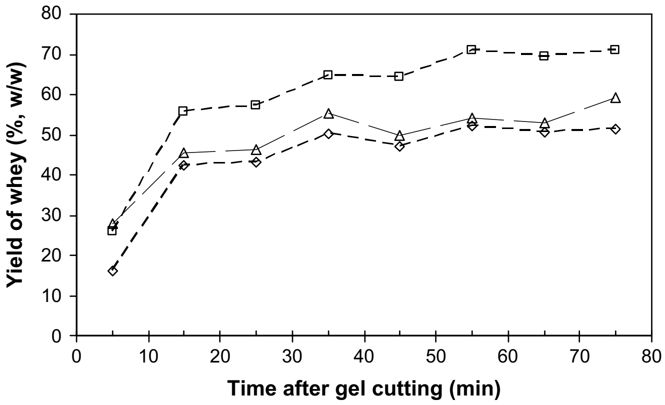

Fig. 1. Production of whey over time during stirring of the curd following gel cutting for typical trials at Low, 0 % (– –□– –), Medium, 2.5 % (——△ —) and High, 5 % w/w (– –◇– –) fat levels.

Dairy Board, Dublin, Ireland), distilled water and cream at 42 °C while being stirred at 44 rpm. $CaCl_2 \cdot 2H_2O$ was added at 2.04 mM to the milk. The milk was cooled to 8 °C and held overnight under gentle agitation (10 rpm).

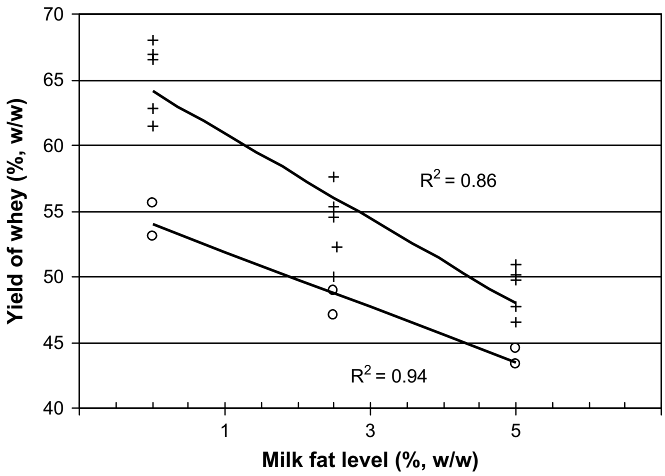

Fig. 2. Yield of whey, Y_w, *vs.* milk fat level, FL_m, at two stages of syneresis, i.e. 15–25 (○) min and 35–75 (+) min, respectively, after gel cutting. At each fat level, seven sampling points are shown, taken at 10 min intervals. Each point represents the mean of three replicates at a single sampling time. Comparison of the slopes at each stage of syneresis shows that they are not statistically different.

2.3. Clotting of milk

On the day following milk preparation, the milk was heated to 32 ± 0.1 °C by water circulation in a heating jacket using a water bath (Grant model Y28, Grant Instruments Ltd., Cambridge, UK) while being stirred at 22 rpm. Milk pH was adjusted to 6.5 using HCl solution (1 M). Approximately 30 mL of milk was taken from the cheese vat for compositional analysis by MilkoScan (MilkoScan 605, Foss Electric, Hillerød, Denmark) to determine fat, protein and lactose contents. Rennet (CHY-MAX extra, EC 3.4.23.4, isozyme B, 600 IMCU mL^{-1}, Chr Hansen Ireland Ltd., Cork, Ireland) was added to the milk (0.18 g kg^{-1} of chymosin in milk) in the cheese vat while being stirred constantly at 31 rpm. Stirring was stopped after 3 min and the stirrers were replaced with twin cutting blades in preparation for cutting.

2.4. Determination of gel firmness and cutting procedure

A 13 mL sample of milk was removed from the cheese vat 3 min after rennet addition and immediately transferred to a small amplitude oscillatory rheometer (Bohlin CVO Rheometer, Bohlin Instruments Ltd., Cirencester, UK), which was pre-heated to the required temperature (32 ± 0.1 °C) for determining the gel cutting time (t_{cut}) at the appropriate firmness level (5, 35 or 65 Pa) (Everard et al., 2008) following the experimental design. When the elastic modulus, G', reached the firmness level required, cutting of the gel was initiated ($t = 0$ min). A fixed cutting programme consisting of 8.3 revolutions of the cutting blades was used to carry out the gel cutting. After gel cutting, the cutting blades were substituted with the twin stirrers in order to agitate the curd/whey mixture. The mixture was stirred at 16 rpm beginning at $t = 4$ min.

Samples (180–270 mL, depending on the analysis required) of the curd/whey mixture were taken using an on-line sampler, at defined intervals, while the curd was being stirred, up to 75 min after gel cutting.

2.5. Measurements of syneresis indices

Yield of whey (Y_W) was calculated as the percentage of whey drained immediately from each sample that was collected. Whey fat (F_W) was measured by the Rose-Gottlieb method (IDF, 1987). Casein fines in whey (C_W) and curd moisture content (M_c) were determined by drying in an oven overnight at 102 °C. Y_W and M_c were determined at 10 min intervals, while C_W and F_W were determined at 20 min intervals.

As the initial value of curd moisture content (at $t = 0$ min) was equal to the water content of the milk, which depended on the fat level in our experiment, an additional parameter involving curd moisture content relative to that of the milk was calculated, as follows.

Table 1
Models for prediction of syneresis indices using multiple linear regression

Syneresis indices[a]	Standardised coefficients (Student's *t* values of the parameters in the model)[b]								
	FL_m	t	$\log t$	t_{cut}	$t_{cut} - t_g$	Adj R^2	SEy[c]	SD[c]	DF[c]
Curd moisture content, M_c	−0.87 (−56.9)	–	−0.42 (−27.7)	–	0.07 (4.72)	0.95	0.92	4.11	212
M_c standardised,[d] M_{cm}	−0.68 (−29.2)	–	−0.63 (−27.3)	0.10 (4.30)	–	0.89	1.06	3.11	212
Fat in whey, F_w	0.92 (21.9)	−0.22 (−5.17)	–	0.09 (2.11)	–	0.88	0.05	0.14	68[e]
Yield of whey, Y_w^2	−0.49 (−14.4)	–	0.72 (21.4)	–	–	0.76	5.96	13.2	213
Casein fines in whey, $\log C_w$	–	–	–	0.72 (10.5)	–	0.51	0.23	0.24	104

[a] Units of syneresis indices: M_c (%, w/w), M_{cm} (%, w/w), F_w (%, w/w), Y_w (%, w/w) and C_w (mg kg^{-1} of whey).
[b] Parameters in the model: cutting time at required firmness level, t_{cut}; time after gel cutting, t; milk fat levels, FL_m; time after set-to-cut time ($t_{cut} - t_g$).
[c] Standard error of prediction, SEy; standard deviation of the response variable, SD; degrees of freedom, DF.
[d] Curd moisture content standardised to water content of milk.
[e] The F_w model was fitted for two levels of fat (2.5% and 5.0%, w/w) because fat level in whey was not measured when $FL_m = 0\%$, w/w.

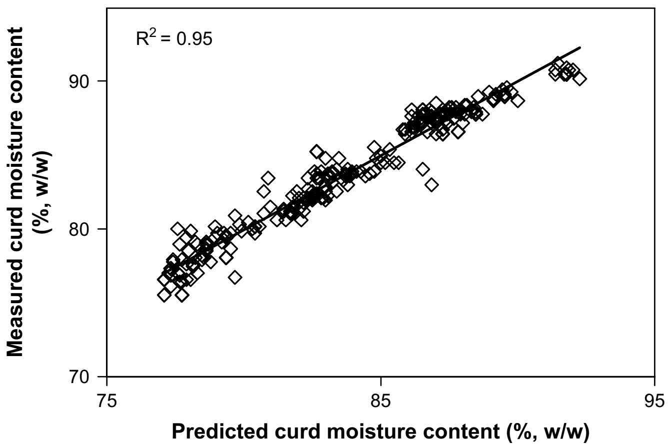

Fig. 3. Measured *vs.* predicted curd moisture content, M_c, for samples taken at 10 min intervals from $t =$ 5 up to 75 min after gel cutting using a linear model, M_c (FL_m, log t, $t_{cut} - t_g$), $N = 216$, cf. Table 1.

Curd moisture content standardised to water content of milk, M_{cm}, was defined as,

$$M_{cm} = 100M_c/(100 - TS_m) \quad (1)$$

where TS_m represented the total solids content of the cheese milk (%, w/w), based on the formulation.

2.6. Statistical analysis

Multiple linear regression was used in this study to determine the significant factors to include in models for predicting syneresis-related parameters using SigmaStat software (Version 3.1, Systat Software Ltd., London, UK) and Minitab-15 statistical software (Minitab Ltd., Coventry, UK).

3. Results and discussion

3.1. Influence of milk fat concentration on the production of whey

Fig. 1 shows that the majority of whey production in the cheese vat occurs while the curd is being stirred during a 15 min period following gel cutting. The trend of whey production is typically first order; when fitted to an equation of the form,

$$Y_w = Ae^{-kt} + B \quad (2)$$

the average R^2 was 0.91.

The production of whey is clearly higher at 0% fat than at the other levels of fat in milk. As observed in Fig. 2, the volume of whey produced decreases with increasing fat concentration in milk at all stages of syneresis. This is in agreement with previous studies under different conditions. Beeby (1959) showed in a small-scale stirred curd setup (38 °C) that whole milk produced less whey than does skim milk. Likewise, Johnston and Murphy (1984) and Marshall (1982), who investigated the effect of fat concentration over a comparable range (0–8%, w/w) but in beaker studies (renneted at 29–30 °C and unstirred curd), found that fat impaired syneresis. The variation in the amount of whey produced in the latter study was comparable with our results, which showed a drop of ~25% in yield of whey when fat level increased from 0% to 5% (w/w).

It needs to be remembered that in our study, a change in fat level in the milk was accompanied by an opposite change in water content, as protein was kept constant. The results of this work need to be understood in this context.

3.2. Developing models for predicting syneresis indices

Models were developed for several syneresis indices to find relationships with milk composition and other cheese-making factors using recombined whole milk with a range of milk fat from 0% to 5.0% (w/w) and a range of gel cutting time, t_{cut}, from 17 to 78 min. Multiple linear regression was used to obtain models for predicting curd moisture, M_c, milk-standardised curd moisture, M_{cm}, yield of whey, Y_w, fat in whey, F_w, and casein fines in whey, C_w, using two experimental variables (fat level in milk, FL_m, and gel firmness at cutting, G') in conjunction with other parameters, i.e., set-to-cut time ($t_{cut} - t_g$), time from gel setting to gel cutting, and time after gel cutting (t) (Table 1). The parameter t_{cut} was substituted for G' where it gave a better prediction, which may have been due to minute differences in conditions between the cheese vat and the rheometer. t_{cut} involves the history of the process in the vat from rennet addition to cutting the gel, whereas G' is only a target value used in the rheometer to determine t_{cut}. At the same G' we get different values for t_{cut} (coefficient of variation (COV) ~ 22%). Regression of the experimental data shows that t_{cut} is influenced by gel time, t_g, in addition to G' or by minute changes in milk composition in addition to G', when t_g is omitted from the regression. It appears that factors that influence gelation also have an influence on t_{cut} independently of the firmness at cutting, and that these factors in turn have an influence on syneresis.

In developing a model for predicting M_c a minimum of three parameters were necessary, namely FL_m, logarithm of time after gel cutting (log t) and $t_{cut} - t_g$ in order of decreasing significance. M_c was predicted with $R^2 = 0.95$ (Fig. 3). In this model, FL_m and t had negative effects on M_c, i.e., high level of fat produced a reduction in curd moisture content (Fig. 4). The negative effect of FL_m results from the variation in total solids of milk in the experimental design, due to the fact that protein level was kept constant while fat level was varied.

Before syneresis begins, the milk sets as a gel whose water content equals the original water content of the milk. Theoretically,

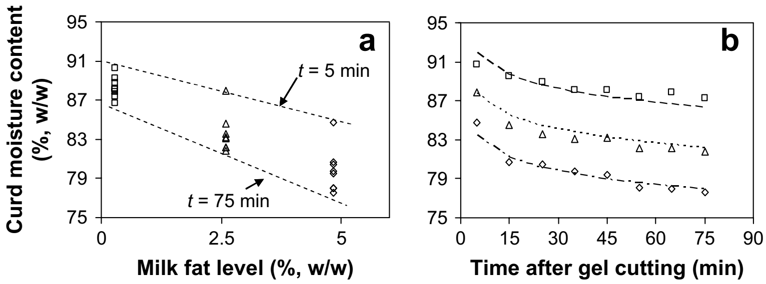

Fig. 4. Influence of milk fat level, FL_m, (a) and time after gel cutting, t, (b) on curd moisture content, M_c, for three typical trials at different FL_m (□ Low, △ Medium and ◇ High fat levels). The dashed lines in (a) indicate sampling times of 5 and 75 min, respectively. The dashed lines in (b) represent predicted curd moisture content using a prediction model M_c (FL_m, log t, $t_{cut} - t_g$) (— — — — Low, - - - - - - - Medium, — - — - — High fat levels) cf. Table 1. The points represent samples taken at eight times during syneresis.

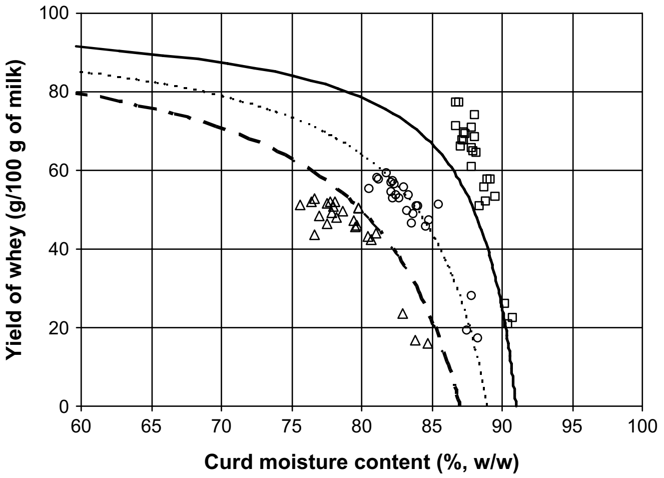

Fig. 5. Relationship between yield of whey and curd moisture content, based on mass balance for the initial conditions in our study, for the fat levels in milk (□ Low,○ Medium, △ High) in our study with measured data superimposed (▬▬▬▬ Low, - - - - - - - Medium, ▬ ▬ High fat levels). The model assumed a value of 6 g/ 100 g for total solids in whey. The values measured for this parameter in the study ranged from 5.4 to 6.8 g/100 g.

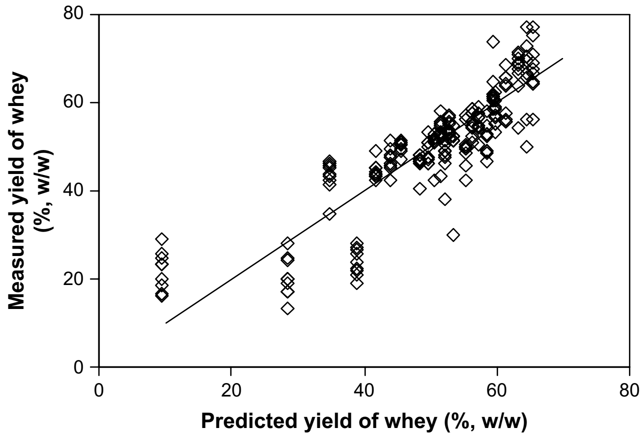

Fig. 7. Measured *vs.* predicted yield of whey, Y_w, for samples taken at 10 min intervals from $t = 5$ up to 75 min after gel cutting based on a linear model, Y_w^2 (log t, FL_m), $N = 216$, cf. Table 1.

this is the starting condition of the cheese curd. As such, at the initial stage of syneresis ($t = 0$), the curd has less water (i.e., curd moisture is lower) at higher fat concentration (cf. the upper dashed line in Fig. 4).

There is an apparent paradox regarding the effect of fat level in milk (FL_m) on final curd moisture in the vat (M_{cf}) and on yield of whey at draining (Y_{wf}): namely as FL_m increases, M_{cf} decreases and Y_{wf} also decreases. This behaviour can be predicted from a mathematical model of syneresis, based on mass-balance. The relationship between yield of whey and curd moisture content is highly non-linear and critically dependent on the starting point (M_{co}), which results in lower yields of whey from the higher fat milks (Fig. 2) being associated with higher reductions in curd moisture content (Fig. 4a). If M_{cf} were a fixed value, i.e., that the curd was drained at a fixed moisture level, the yield of whey would decrease with increasing FL_m (i.e., with decreasing M_{co}). It can be shown mathematically that the extent of the change in Y_w (with FL_m) depends on the value chosen for M_{cf}, being very pronounced for a high value of M_{cf} (approaching M_{co} which is 87 for the high fat level, i.e., no production of whey) (Fig. 5). For example, if $M_{cf} = 84$, then Y_{wf} decreases (from 69 to 30%, w/w of milk) with increasing FL_m (from 0% to 5%).

If on the other hand, we hypothesise a fixed reduction, ΔM_c, in curd moisture content during syneresis in the vat (i.e., ΔM_c is independent of FL_m, let us say, $\Delta M_c = 8\%$, w/w of curd), Y_{wf} also decreases (from 72 to 53 g 100 g^{-1} of milk) with increasing FL_m (from 0% to 5%). In an attempt to compensate for the variation in initial water content of the curd (since at $t = 0$, water content in curd ~ water content in milk), we developed a model for M_{cm}, the curd moisture content standardised to water content of milk, to determine if fat level in milk has a net effect on curd moisture content. The model predicted M_{cm} with $R^2 = 0.89$ involving three factors namely FL_m, log t and t_{cut} in decreasing significance (Fig. 6). Fat level in milk had a major effect in this model, showing that FL_m reduced curd moisture content over and beyond its direct effect on initial curd moisture content. There is a large trend with time, as representing by log t.

To determine a regression model for Y_w, it was necessary to transform Y_w to Y_w^2 to satisfy the assumptions behind statistical significance in linear regression, i.e., normality of residuals and homoscedasticity. The model developed for predicting Y_w ($R^2 = 0.76$) involves two factors namely log t and FL_m in order of

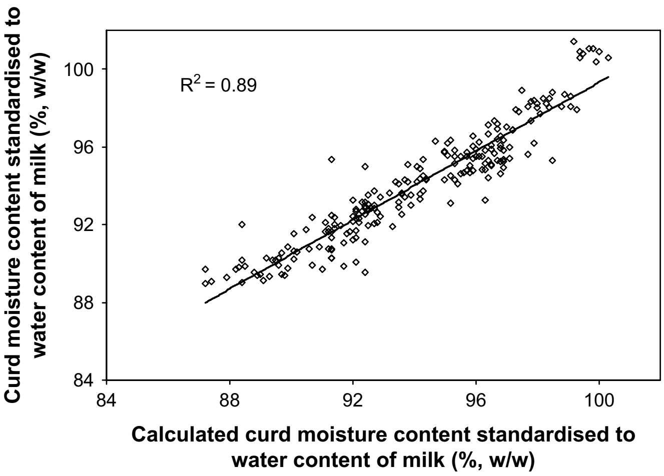

Fig. 6. Calculated *vs.* predicted curd moisture content standardised to water content of milk, M_{cm}, determined according to Eq. (1), for samples taken at 10 min intervals from $t = 5$ up to 75 min after gel cutting using a linear model, M_{cm} (FL_m, log t, t_{cut}), $N = 216$, cf. Table 1.

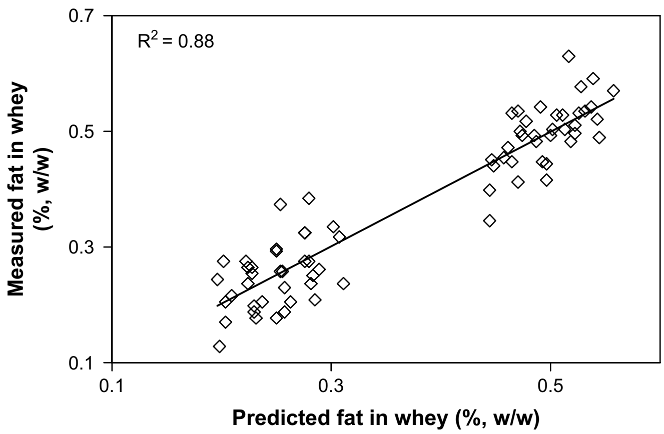

Fig. 8. Measured *vs.* predicted fat in whey, F_w, for samples taken at 20 min intervals, between $t = 15$ and $t = 75$ min after gel cutting using a linear model, F_w (FL_m, t, t_{cut}), N = 72, cf. Table 1. Fat in whey was measured at two levels of FL_m i.e., 2.5 and 5.0 % w/w. The straight line ($Y = X$) indicates predicted values based on the model, allowing a visual comparison with measured values.

decreasing significance (Fig. 7). This simple model shows a logarithmic, i.e., non-linear, effect of time on yield of whey. FL_m decreases the production of whey in accordance with many authors (van Dijk, 1982; Marshall, 1982). This is thought to be due to the clogging effect of the fat globules in the casein matrix, impeding the outward flux of whey from curd particles (Dejmek and Walstra, 2004; Guinee, Mulholland, Kelly, and O'Callaghan, 2007). However, Y_w increases with t during cheese manufacture due to mechanical processes (gel cutting, curd stirring and pressure dynamics) and physical changes such as contraction of the curd in accordance with Everard et al. (2008), who varied curd stirring speed and gel cutting intensity.

F_w was predicted with $R^2 = 0.88$ using three parameters FL_m, t and t_{cut} in order of decreasing significance (Fig. 8). The positive effect of FL_m in this model means an increment of fat losses in serum with increasing FL_m in accordance with Fenelon and Guinee. (1999). The small positive effect of t_{cut} in this model is consistent with Riddell-Lawrence and Hicks (1989). The C_w model involves a logarithmic transformation of C_w to satisfy the assumptions behind linear regression, i.e., normality and constant variance tests. This model predicted log C_w with $R^2 = 0.51$ involving one factor, i.e., t_{cut}. While this model is not good enough for prediction, it shows a clear positive influence of t_{cut} on casein fines. This finding relates to the range of coagulum strength in our study, as a very soft cutting curd would also increase loss of curd fines (Thomasow and Voss, 1977).

A previous study on the influence of milk composition on cheese characteristics, also carried out under constant protein levels in milk, gave trends similar to those in our study with respect to the effect of fat level in milk upon moisture in cheese, showing that the effects found in this study with regard to curd moisture carry through to cheese (Guinee et al., 2007).

4. Conclusions

The present study shows that milk fat concentration, at constant protein level, has a great influence on syneresis indices, i.e., M_c, M_{cm}, Y_w and F_w. A clear effect of gel firmness on casein fines was observed. The best overall prediction, in terms of coefficient of determination, was for moisture content based on fat level in milk, time after gel cutting and set-to-cut time. This work provides understanding of the way that milk standardisation with respect to milk fat level gives better control of curd moisture in cheese manufacture. The prediction models developed in this work can be used for better control of syneresis during curd stirring and of curd moisture at draining. Better understanding of syneresis in the vat can contribute to control of dry matter in cheese, which assists in meeting legislation requirements, optimising quality and maximising profitability. Control of curd moisture at drainage can also assist with consistency of territorial cheeses.

Acknowledgments

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