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Comparison between Formagraph and low-amplitude oscillation rheometry in monitoring coagulation properties of acid induced gels in bovine milk

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ABSTRACT

Acid coagulation properties of milk (gelation time, gel-firming rate and maximum gel firmness) were analysed simultaneously by Formagraph and LAOR. Good agreement between acid coagulation data by the two methods was perceived. Results from this investigation shows that Formagraph can be used in acid coagulation studies when analysing a larger set of milk samples.

INTRODUCTION

Preparation of acid milk protein gels involve the structural destabilization of casein micelles through decrease in pH. In milk, the steric repulsive forces caused by the outer hairy structure of κ-CN and the hydrophobic binding via colloidal calcium phosphate from the interior of the micelles accounts for the stability of casein micelles[1]. During acidification, the steric repulsive force is reduced by an increase in the positive charges while the colloidal calcium phosphate become more soluble and it is released from the interior of the micelles[2].

Several factors are known to affect coagulation properties of milk. For example, temperature history of the milk, pH, enzyme concentration[3], genetic polymorphism[4,5] and feeding regime[6]. Heat treatment of milk at higher temperature leads to whey protein denaturation[7]. The denatured whey proteins interact with caseins through their hydrophobic sites[8], which improve the texture and strength of yoghurt gels.

Milk gelation properties are determined by gelation time, gel strength and gel-firming rate. These parameters plays an important role in order to optimize the production of fermented milk products at large scale. Several methods are used to evaluate gelation or coagulation properties of milk. For example, Formagraph is used to measure rennet coagulation[9,10,11]. The Formagraph can analyse many samples at the time. Low-amplitude oscillation rheometry (LAOR)[4,12] is the conventional method used to measure acid coagulation properties of milk, but only one sample can be analysed at the time. Optical methods, as for example, Fourier transform infrared spectroscopy has also been used[13]. Several studies have been used to compare rennet coagulation properties between Formagraph and LAOR[14,15]. To our knowledge, there is no study, which has evaluated the acid coagulation properties between Formagraph and LAOR. As LAOR is time consuming and is limited to one sample at the time, a more effective method is needed for a larger number of samples. Hence, the current study was anticipated to evaluate if the

Formagraph could be used to give reliable results on acid gelation properties.

MATERIALS AND METHODS

Milk samples

The methods of gel characterisation were tested in two steps. In the first step, (four-sample test) individual milk samples from four cows were analysed in quintuplet by Formagraph and Small amplitude oscillation rheometry to test the repeatability within each sample. In the second step, (ten-sample test) individual milk samples were analysed in duplet by Formagraph and once by LAOR to test if the two methods showed a similar variation between different samples.

Four-sample test: Individual morning milk samples from four (4) lactating cows were collected in two consecutive weeks, and samples from two cows were analysed in each week.

Ten-sample test: Ten (10) samples from ten cows were collected and analysed the same week.

All experimental animals were kept in the same housing and feeding management at the Centre for Animal Research (SHF) of Norwegian University of Life Sciences in Ås, Norway. The milk samples from 10-sample test were analysed once in triplicate for total protein and casein by using MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark) as described by Inglingstad et al [6]. Before acidification, milk samples were transferred into 15mL Falcon tubes and heat treated at 95^{0}C for 5 minutes in a temperature controlled water bath and then cooled to 32^{0}C in ice water.

Formagraph analysis

Acid coagulation properties were monitored by Formagraph (LAT; Foss-Italia, Padova, Italy). The working principle of the apparatus has previously been described [11,14,16]. 3% Glucono-δ-lactone (GDL) was added into the wells of the Formagraph blocks followed by addition of 10 mL of milk samples maintained at 32^{0}C. The mixture was mixed simultaneously by using the Formagraph multiple spoon for approximately 30 seconds and transferred to the Formagraph recording system. Parameters recorded in the Formagraph were Formagraph acid Gelation Time (FGT), defined as the time interval in minutes from start acidification to the time at which the width of bifurcate was increased to 1.2 mm; Formagraph Maximum Gel Firmness, (FMGF) defined as the point with the maximum width in mm of the bifurcate. Formagraph Gel-firming rate, mm/min (FGFR) defined as the slope of width of the curve *vs.* time curve (Figure 1). The width of the bifurcate as a measure of gel development was measured at 15 seconds interval for 60 minutes.

Rheometry analyses

Rheological analyses on the acidified milk gels was monitored simultaneously by using Paar Physica universal dynamic spectrometer (MCR 300, Anton Paar) equipped with a bob-cup measurement system (CC27/*Ti* with diameter 26.657 mm and 40.03 mm length: bob specifications and C-CC27/T200/*Ti* with 28.926 mm diameter: cup specifications). 14mL of milk was acidified with 3% GDL and shaken vigorously for 30 seconds. The mixture was transferred into the measuring system maintained at 32^{0}C with angular frequency of 10 rad/sec at a constant strain of 0.1% within the linear visco-elastic range (LVR).

The following parameters were recorded on the LAOR, gelation time (GT), which was defined as the time in minutes when G'≥1Pa [17]. Maximum gel firmness (MGF), defined as the maximum gel strength attained measured in Pa and gel-firming rate (GFR'), defined as the slope of G' *vs*. time curve (Pa/min) [15]. Storage modulus (G') as a measure of gel strength was recorded at 45 seconds interval for 60 minutes.

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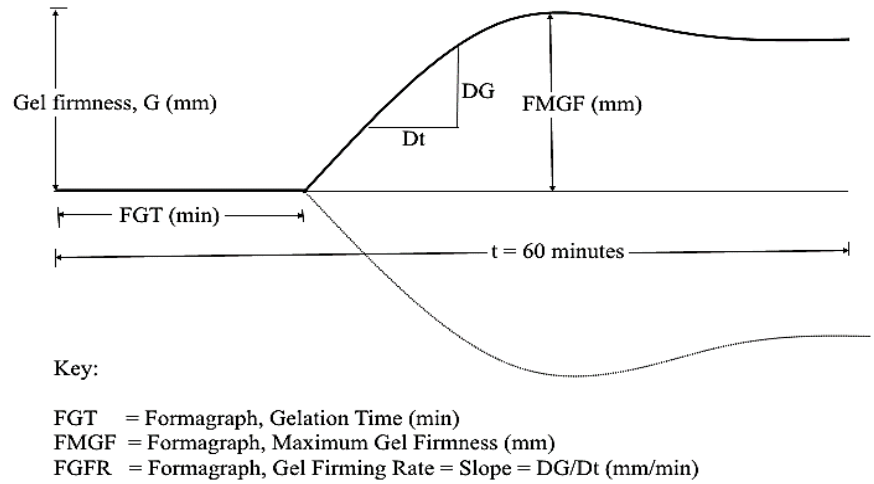

Figure 1: Acid coagulation parameters as analysed by Formagraph

Statistical analysis

The regression procedure of Statistical Analytical Software (SAS) was carried out to test the relationship between acid coagulation properties (GT *vs.* FGT, GFR *vs.* FGFR and MGF *vs.* FMGF) on the four and ten samples test between Formagraph and LAOR.

RESULTS

The four-sample test aimed at assessing the repeatability of the acid coagulation results between the two methods.
The ten-sample test intended to assess the differences between the samples analyzed by the two methods.

Four-sample test

The standard deviation (SD) within each sample in the four-sample test showed that the repeatability was similar between the two methods (Fig. 2, 3 & 4).

Regression analysis on the four sample test shows weaker correlation coefficients in all variables tested (Table 1), compared to the ten sample tests (Table 2).

Table 1: Regression analysis of the acid coagulation data as analysed by Formagraph and LAOR

Variable	n	R^2	CV	*p*
GT vs. FGT	20	20.99%	7.93	0.0422
GFR vs. FGFR	20	48.00%	13.78	0.0007
MGF vs. FMGF	20	43.00%	9.96	0.0017

Despite of the weak correlation coefficients (Table 1) in the four-sample test, there is a sound agreement in gelation time between the two methods, (Figure 2). Rheometry analyses showed shorter gelation time in average compared to the Formagraph. These results show that the LAOR as a non-destructive type of measurement has a higher sensitivity compared to the Formagraph. Hence, it takes shorter time for the instrument to detect gel development.

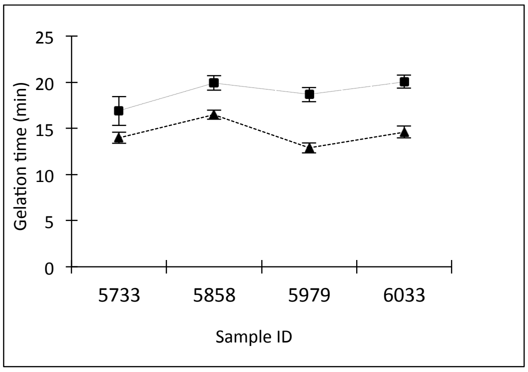

Figure 2: Means with standard deviation (SD) for gelation time between Formagraph (□) and LAOR (□)

Figure 3 discloses the pattern for the gel-firming rate by the two methods investigated. A similar pattern of gel-firming rate was achieved by both methods, where higher gel-firming rate was perceived in sample 6033 compared to 5858 in both the Formagraph and the LAOR.

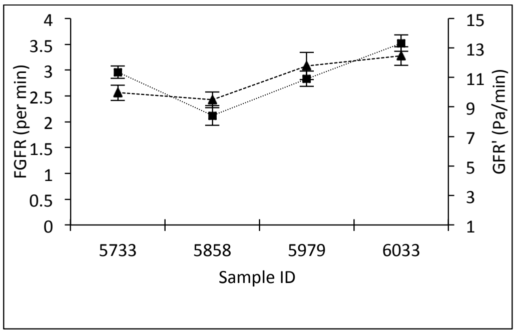

Figure 3: Means and SD for the gel-firming rate between Formagraph (□) and LAOR (□)

The results for the maximum gel firmness for the two instruments tallies to each other, as shown in Figure 4. Both the Formagraph and LAOR showed a similar trend for maximum gel firmness.

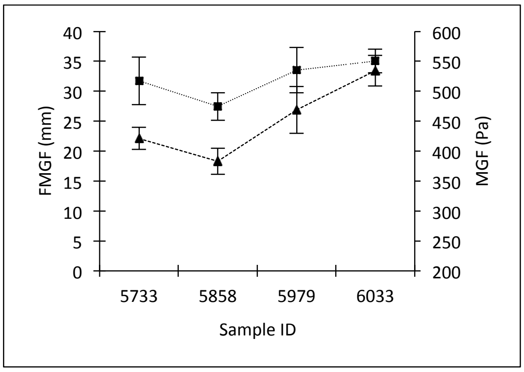

Figure 4: Means and SD for the maximum gel firmness between Formagraph (□) and LAOR (□)

Ten-sample test.

The ten-sample test aimed to verify the similarity of the methods by studying the steadiness of the results between samples analysed by the two methods. Sample 6033 showed higher total protein content (4.10%) and casein (3.05%) compared with sample 5616 with mean 3.25% and 2.48% for total protein and casein respectively.

Table 2 shows the regression analysis when comparing the formagraph and LAOR data was achieved between the methods on Gelation time and Gel-firming rate respectively.

Table 2: Regression analysis of the acid coagulation data as analysed by Formagraph and LAOR

Variables	n	R^2	C.V	p
GT *vs*. FGT	10	81.2%	10.21	0.0004
GFR *vs*. FGFR	10	83.57%	11.37	0.0002
MGF *vs*. FMGF	10	42.03%	21.52	0.043

Figure 5 shows the pattern for the gelation time in ten samples between the two methods. Reliable agreement on acid gelation time between the two methods was noticed as in the four-sample test.

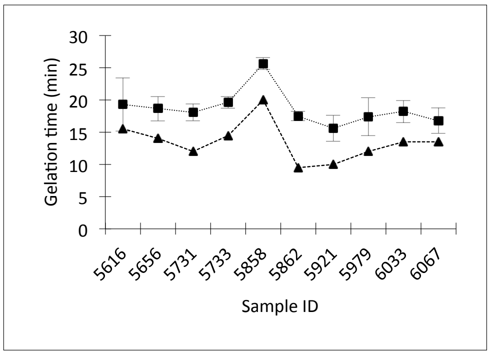

Figure 5: Gelation time pattern between Formagraph (■) and LAOR (▲).

As in the four-sample test, good agreement on the gel-firming rate between the two methods was observed in the 10-sample test, as illustrated in Figure 6. Sample 5858 showed a slower rate of gel formation compared to other samples by both methods.

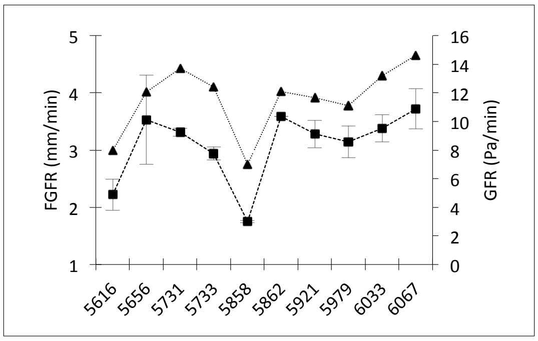

Figure 6: Gel-firming rate pattern between Formagraph (■) and LAOR (▲).

A weaker correlation was found between the methods for maximum gel firmness (R^2=42.03 %) compared to the other variables in the 10 sample trail (Table 2). However, the correlation was comparable to that obtained in the four-sample test. However, in the four-sample test, the SD between the samples were quite large for the maximum gel firmness compared to the SD of the gelation time and gel-firming rate. Therefore, the lower correlation between the methods on maximum gel firmness could be expected.

Maximum gel firmness analysed in the 10 sample-test (Figure 7) showed a similar agreement between the two methods as in the 4-sample test. For example, sample 5858 showed low gel strength in each method as expressed in the four and ten-sample test. However, some samples also showed an opposite trend with the two methods, i.e. sample 5731 and 5733.

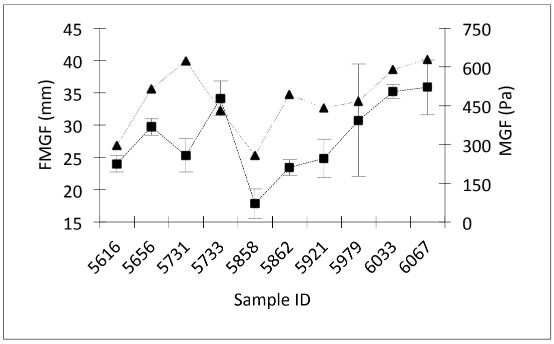

Figure 7: Maximum gel firmness between Formagraph (■) and LAOR (▲)

CONCLUSION

Comparable results for the acid coagulation parameters were obtained for gelation time and gel-firming rate between Formagraph and Small amplitude oscillation rheometry, and our results shows that Formagraph can be used as an alternative method for analyzing the acid coagulation properties of milk on large sample sizes.

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