Monitoring milk coagulation process by near infrared spectroscopy

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Introduction

Milk coagulation is an important step in cheese making. The mechanisms involved and the effects of some parameters (pH, temperature, calcium concentration) on coagulation of casein fractions are quite well known.[1,2]

Different methods for monitoring milk protein changes were developed in order to control the coagulation process either to understand the aptitude of the milk to coagulate[3] or to check when the appropriate gel texture is reached to cut the curd.

Two principal methods were used to study these phenomena: rheological determinations[3,4] and thermal methods.[5]

Near infrared (NIR) was used to monitor milk coagulation for the determination of the curd cutting time, using both specified wavelength[6,7] and full-spectrum information.[8]

In a previous work,[9] it was found that NIR could be used to monitor the critical events taking place in the primary phase of coagulation. In particular, interesting results were obtained plotting 2nd derivative absorbance values at 1928 nm against time: this established the characteristic coagulation trend of milk, related to the evolution of the equilibrium between water, as the active component and the other constituents during the phase changes.

On the basis of these preliminary results, this work was aimed to prove the feasibility of NIR spectroscopy to detect the critical events during the milk coagulation process, also working under different conditions and using different kinds of substrates. This step should be developed and realised to guarantee the minimum number of experiments required to demonstrate the future possibility of designing an appropriate and simple in-line instrument to monitor milk coagulation, on the basis of a proven relationship between flock formations and water measurements.

Materials and methods

Forty-six different coagulation tests were carried out in duplicate at 35°C. Different kinds of skimmed milk powder and liquid milk (whole raw, defatted raw and pasteurised) were used as substrate (Table 1) and two liquid rennet (containing 22% of bovine pepsin, strength = 1 : 14,600 Soxhlet units, Chr. Hansen, Corsico, Italy) solutions (0.8, 1.6% in distilled water) were added to milk at 2%.

Skimmed milk samples were reconstituted at 10% of solids content (w/w) in $CaC1_2$ 0.05% before the analysis. Defatted samples were obtained from whole raw milk samples by centrifugation at 6,000 rpm for 15 minutes. Pasteurised samples, traded by five different Italian companies, were used for these experiments.

Calf rennet concentrations were chosen on the basis of rennet concentrations used for Formagraph measurements.

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Table 1. Milk samples analysed after addition of two different amounts of calf rennet.

RECONSTITUTED MILK–CODE	LIQUID MILK (Italy)–CODE
Skim Milk Powder 1 (France)–SMP1	Raw whole milk (bulk of ILC farm)–M1
Skim Milk Powder 2 (France)–SMP2	Raw whole milk (bulk of ILC farm)–M2
Skim Milk Powder 3 (Austria)–SMP3	Raw whole milk (bulk of commercial Company)–M3
Skim Milk Powder 4 (NL)–SMP4	Raw whole milk (bulk of commercial Company)–M4
Skim Milk Powder 5 (NL)–SMP5	Raw defatted milk–SM1
Skim Milk Powder 6 (NL)–SMP6	Raw defatted milk–SM2
Skim Milk Powder 7 (NL)–SSMP7	Raw defatted milk–SM3
Skim Milk Powder 8 (Vietnam)–SMP8	Raw defatted milk–SM4
Skim Milk Powder 9 (Germany)–SMP9	Pasteurised milk–PM1
Skim Milk Powder 10 (Germany)–SMP10	Pasteurised milk–PM2
	Pasteurised milk - PM3
	Pasteurised milk - PM4
	Pasteurised milk - PM5

The clotting time was calculated by using both a Formagraph instrument (Foss-Electric, Hellerup, Denmark) and visual observation of the formation of the first flocks.

The NIR spectra were recorded with a holographic grating spectrometer (Bran+Luebbe InfraAlyzer 500), from 1100 to 2500 nm at 4 nm intervals (351 data points).

Light absorption was expressed as log (1/*R*) values. Measurements were carried out in transflectance mode. The instrument was equipped with a thermostatable liquid sample cell. Temperature was controlled by an external circulating bath (Haake, mod. F3-CH). Milk samples were injected into the cell at 35°C and then the rennet was added. Spectra were recorded in sequence every 70 seconds after rennet addition. Data were processed by plotting 2nd derivative absorbance values at 1928 nm vs time.[9] Spectroscopic clotting time (CT) was calculated as the maximum value detected on the curves.

All data shown in the Figures are the average of two replicates of lab coagulation tests carried out on the same day.

Results and discussion

In order to compare spectra within each group of samples, data were centred on the average values calculated by processing all data related to each group.

Skimmed milk powder

Tests carried out by using skimmed milk powders from different origins as the substrate (Figures 1 and 2) confirmed the feasibility of NIR to detect changes during the milk coagulation process. In all cases NIR was able to detect the first flock formation according to the visual observation of this phenomenon and about two to four minutes before the coagulation time measured by Formagraph (Table 2). The clotting time (CT), interpolated by using NIR 2nd derivative at 1928 nm, proved the relationship between the amount of added rennet for the same sample. At 0.8% rennet concentration, the clot-

Table 2. Clotting measurement times.

Samples	CT	Visual observation	Formagraph
SMP1 – 0.8	23′ 30″	23′ 00″	24′ 43″
SMP2 – 0.8	21′ 00″	20′ 12″	23′ 32″
SMP3 – 0.8	22′ 16″	21′ 32″	24′ 58″
SMP4 – 0.8	23′ 30″	22′ 25″	26′ 00″
SMP5 – 0.8	23′ 30″	22′ 59″	26′ 14″
SMP6 – 0.8	22′ 17″	22′ 44″	25′ 12″
SMP7 – 0.8	21′ 00″	19′ 31″	22′ 45″
SMP8 – 0.8	21′ 00″	19′ 32″	22′ 57″
SMP9 – 0.8	23′ 30″	23′ 07″	26′ 33″
SMP10 – 0.8	23′ 30″	23′ 59″	25′ 59″
SMP1 – 1.6	16′ 30″	15′ 55″	18′ 27″
SMP2 – 1.6	12′ 07″	11′ 02″	13′ 27″
SMP3 – 1.6	12′ 07″	11′ 21″	13′ 54″
SMP4 – 1.6	13′ 23″	12′ 34″	15′ 56″
SMP5 – 1.6	15′ 17″	14′ 47″	18′ 21″
SMP6 – 1.6	15′ 17″	15′ 20″	18′ 04″
SMP7 – 1.6	08′ 17″	08′ 50″	11′ 45″
SMP8 – 1.6	10′ 50″	09′ 36″	12′ 11″
SMP9 – 1.6~	16′ 33″	16′ 30″	20′ 19″
SMP10 – 1.6	16′ 33″	16′ 38″	20′ 18″
PM1 – 0.8	28′ 00″	27′ 02″	31′ 02″
PM2 – 0.8	26′ 40″	25′ 50″	29′ 11″
PM3 – 0.8	24′ 50″	24′ 48″	28′ 03″
PM4 – 0.8	26′ 40″	25′ 03″	28′ 54″
PM5 – 0.8	24′ 50″	24′ 56″	28′ 12″
PM1 – 1.6	24′ 50″	24′ 58″	26′ 34″
PM2 – 1.6	24′ 50″	23′ 59″	26′ 21″
PM3 – 1.6	22′ 17″	22′ 34″	25′ 14″
PM4 – 1.6	22′ 17″	22′ 57″	25′ 58″
PM5 – 1.6	23′ 33″	23′ 10″	26′ 09″
M1 – 0.8	22′ 17″	21′ 12″	24′ 01″
M2 – 0.8	21′ 00″	20′ 42″	23′ 41″
M3 – 0.8	23′ 33″	23′ 07″	25′ 59″
M4 – 0.8	23′ 33″	23′ 31″	26′ 17″
M1 – 1.6	17′ 50″	17′ 12″	20′ 15″
M2 – 1.6	16′ 33″	16′ 54″	19′ 58″
M3 – 1.6	17′ 50″	18′ 46″	21′ 54″
M4 – 1.6	19′ 09″	19′ 12″	22′ 07″
SM1 – 0.8	24′ 50″	23′ 51″	26′ 50″
SM2 – 0.8	22′ 17″	23′ 13″	26′ 10″
SM3 – 0.8	26′ 07″	25′ 57″	29′ 01″
SM4 – 0.8	27′ 23″	26′ 00″	29′ 28″
SM1 – 1.6	20′ 23″	20′ 12″	22′ 59″
SM2 – 1.6	20′ 23″	19′ 32″	22′ 41″
SM3 – 1.6	21′ 00″	20′ 57″	23′ 21″
SM4 – 1.6	21′ 00″	21′ 43″	23′ 58″

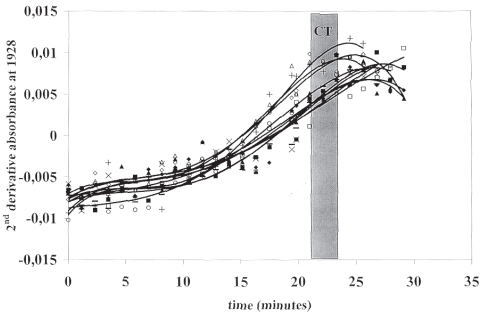

Figure 1. 2nd derivative absorbance at 1928 vs time in reconstituted skimmed milk coagulated by the addition of rennet at 0.8%. (□ = SMP1, = SMP2, X = SMP3, — = SMP4, ♦ = SMP5, ❍ = SMP6, Δ = SMP7, + = SMP8, ■ = SMP9, ▲ = SMP10.

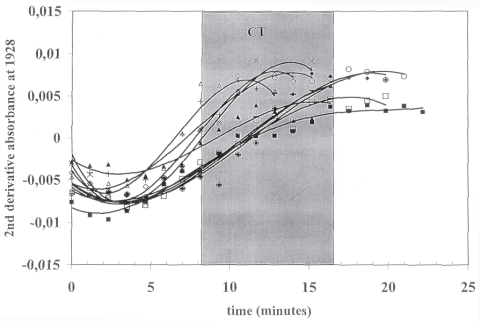

Figure 2. 2nd derivative absorbance at 1928 vs time in reconstituted skimmed milk coagulated by the addition of rennet at 1.6%. (□ = SMP1, = SMP2, X = SMP3, — = SMP4, ♦ = SMP5, ❍ = SMP6, Δ = SMP7, + = SMP8, ■ = SMP9, ▲ = SMP10.

ting time had an average delay of eight minutes compared to the 1.6% rennet concentration. Data also suggested that CT could be related to the technological treatments applied for the production of the different skimmed milk powders tested. Differences of trends were found which were more evident for the series with 1.6% of rennet added.

Raw whole milk and raw defatted milk

Results are reported in Figures 3 and 4. A good relationship between the visual observation of flock formation and NIR data was found, confirming the suitability of 2nd derivative at 1928 in monitoring coagulation process.

Whole and defatted samples were shown to have different trends, suggesting a possible role of fat in the equilibrium of "liquid milk system". Different trends, in particular in the first part of the curve,

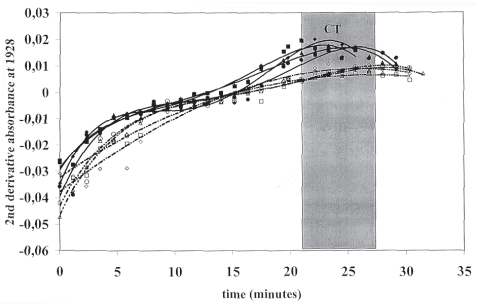

Figure 3. 2nd derivative absorbance at 1928 v. time in raw whole milk and in defatted milk coagulated by the addition of rennet at 0.8%. (◆ = M1, ■ = M2, ▲ = M3, ● = M4, = SM1, □ = SM2, Δ = SM3, ❍ = SM4; — = raw milk, – · – = defatted milk.

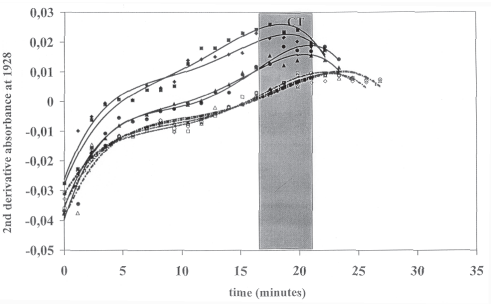

Figure 4. 2nd derivative absorbance at 1928 v. time in raw whole milk and in defatted milk coagulated by the addition of rennet at 1.6%. (◆ = M1, ■ = M2, ▲ = M3, ● = M4, = SM1, □ = SM2, Δ = SM3, ❍ = SM4; — = raw milk, – · – = defatted milk.

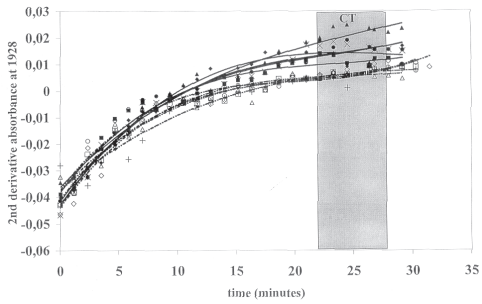

Figure 5. 2nd derivative absorbance at 1928 v. time in pasteurised milk coagulated by the addition of rennet at 0.8% and 1.6% (= PM1 0.8%, □ = PM2 0.8%, △ = PM3 0.8%, ❍ = PM4 0.8%, + = PM5 0.8%, ◆ = PM1 1.6%, ■ = PM2 1.6%, ▲ = PM3 1.6%, ● = PM4 1.6%, X = PM5 1.6%; – · – = rennet 0.8%, — = rennet 1.6%).

were found making a comparison between raw milk (Figures 3 and 4) and skimmed milk (Figures 1 and 2): these data confirm the important role of water as an active component, not only a solvent, and suggest the possibility of studying the different forms of water (free, bound) by using NIR. Differences were detected, in particular, as modification of trends during the first ten minutes, when apparently no changes in aggregation occurred.

However, for raw milk samples, too, both whole and defatted, clotting time was delayed proportionally (four to five minutes) when the amount of rennet was decreased.

Pasteurised milk

Figure 5 shows results obtained for pasteurised milk samples.

The role of fat was not detectable in this group, probably because of the use of homogenisation during the pasteurisation process. These samples showed trends more comparable with raw defatted milk than with raw whole milk samples.

The delay in coagulation due to different amount of rennet was reduced, on average, by two minutes.

As in the other samples, water appears to be one of the principal components involved in the phases changes and, also, in this case, the first part of the curves should be investigated to explain and to verify what kind of rearrangements take place in it.

Conclusions

NIR spectroscopy proved to be a suitable tool to monitor milk coagulation processes and to detect critical events taking place in it also working under different conditions and using different types of substrates.

The use of different amounts of rennet heavily modifies the trends, even within the same group of samples, suggesting further investigations to understand the nature of the events that can be monitored by NIR spectroscopy better, including the role of water as active component.

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