Journal of Dairy Research (2005) **72** 303–310. © *Proprietors of Journal of Dairy Research 2005*
doi:10.1017/S0022029905001020 *Printed in the United Kingdom*

Ultrasonic analysis of rennet-induced pre-gelation and gelation processes in milk

Catherine Dwyer[1], Liam Donnelly[2] and Vitaly Buckin[1,*]

[1] Department of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland
[2] Teagasc, Dairy Products Research Centre, Moorepark, Co. Fermoy, Ireland

Received 23 December 2003 and accepted for publication 16 November 2004

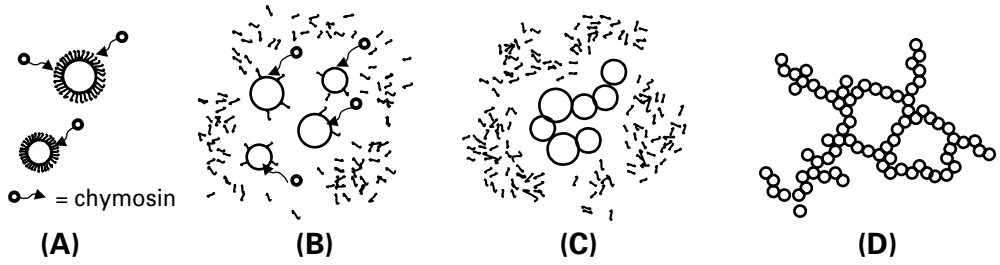
Dynamics of micro-structural changes in milk during the renneting process were analysed using high-resolution ultrasonic spectroscopy in combination with dynamic rheology and NIR transmission measurements. Two independent ultrasonic parameters, velocity and attenuation were measured in the frequency range 2 to 15 MHz, as a function of time after addition of rennet to milk. The results show an initial decrease of 20 nm for the average diameter of micelles caused by hydrolysis of the κ-casein 'hairy' layer followed by an aggregation of the micelles into small clusters (effective aggregation number of 3) and then formation of the gel structure. It was found that evolution of ultrasonic attenuation in the renneting process could well be described by the scattering of the ultrasonic waves on aggregates. The evolution of ultrasonic velocity is well described by the scattering theory but deviates from the predicted curve at the gelation stage of the process, which shows the difference in propagation of ultrasonic waves in a gel structure compared with dispersions. Overall, we found high-resolution ultrasonic spectroscopy to be a powerful tool for analysis of microscopic processes in the formation of milk gel. It allows the characterisation of the pre-gelation processes, such as hydrolysis and aggregation, and the initial stages in the formation of the gel network as well as monitoring of the microscopic evolution in the gel at the post-gelation stage.

Keywords: High-resolution ultrasonic spectroscopy, ultrasonic velocity, attenuation, milk, casein micelles, rennet coagulation.

Rennet-induced coagulation of the casein component of milk to form a gel is the first step of cheesemaking. The casein gel consists of chains of casein micelles linked together in a 3-dimensional network. The casein micelle is the main structural component of milk and has an open, porous structure. The micelle is composed mainly of the casein phosphoproteins, α_{s1}-casein and α_{s2}-casein, β-casein and κ-casein and colloidal calcium phosphate, which plays a key role in 'cementing' the caseins together. The α_{s1}-, α_{s2}- and β-caseins form the hydrophobically charged micelle core. κ-Casein (12–15% total casein) is responsible for the stability of the casein micelle (Fox & Sweeney, 1998). It is located mainly on the surface of the micelle where its hydrophobic N-terminal region links hydrophobically with the calcium-sensitive α_{s1}, α_{s2}- and β-caseins while its hydrophilic C-terminal region protrudes into the surrounding aqueous environment, stabilising the micelles against coagulation by exerting a negative surface charge and steric stabilisation.

On addition of rennet (chymosin) to milk (Fig. 1A), κ-casein is hydrolysed specifically at the Phe_{105}–Met_{106} bond at the junction between the para-κ-casein and glycomacropeptide moieties (Dalgleish, 1993). The hydrophilic macropeptides (about 30% of κ-casein, i.e. 4–5% of total casein) diffuse into the serum while the para-κ-casein remains attached to the micelle. Removal of the macropeptides from the surface of the casein micelles, which play a role of steric stabilising layer, destabilises the colloid (Payens, 1977, Dalgleish, 1979). The proteolysis of κ-casein by chymosin is referred to as the primary phase of rennet coagulation (Fig. 1B). When a significant amount of the total κ-casein in milk has been hydrolysed, around 86% according to Dalgleish (1979) and Green et al. (1978), the colloidal stability of the micelles is reduced and they coagulate (Fig. 1C). Calcium ions are essential for the coagulation of rennet-altered micelles. A gel network is formed from the coagulated micelles, which undergoes progressive re-alignments and re-arrangements over time (Fig. 1D).

*For correspondence; e-mail: buckinv@eircom.net



(A) Casein micelles (intact κ-casein 'hairy' layer) on initial introduction of rennet (chymosin) to the milk

(B) Casein micelles partially denuded of κ-casein: Ultrasonic velocity & attenuation measurements

(C) Exensively denuded micelles in process of progressive aggregation into gel network: Ultrasonic velocity, attenuation, NIR measurements

(D) Progressive gel network formation: Ultrasonic velocity, attenuation, rheology, NIR measurements

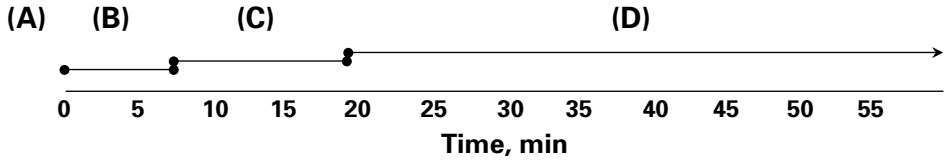


Fig. 1. Illustration of the recognised renneting process in milk and comparison of ultrasonic measurements with other techniques for monitoring this process in milk at 30 °C (Dalgleish, 1993).

Understanding and control of the gelation process requires techniques for monitoring the micro-structural changes at all stages of pre-gelation and gelation. Unfortunately, most traditional techniques either cannot follow all stages of the process (e.g. dynamic rheology detects the formation of the gel network but is not sensitive to pre-gelation processes) or do not provide detailed micro-structural information (e.g. limitations caused by optical non-transparency and multiple scattering effects in optical techniques). An objective technique that can be used for non-destructive analysis of the micro-structural changes in the milk system during the first step of cheesemaking, in particular those that occur during the hydrolysis and subsequent pre-gelation processes, would be an important tool from both a research and a commercial perspective. This technique would aid in controlling and standardising the coagulation process, which would in turn allow producers to have more control over cheesemaking practice.

A technique with significant potential in analysis of these changes in milk is high-resolution ultrasonic spectroscopy. This technique based on the measurements of parameters of low-intensity, non-destructive ultrasonic waves propagating through analysed samples. Ultrasonic waves have the ability to propagate through optically opaque materials and therefore are highly suited for use in milk studies. Measurements of ultrasonic parameters, velocity and attenuation, have been carried out in food systems and these parameters have been shown to be sensitive to composition, microstructure and phase transitions in different foods (McClements et al. 1993; McClements, 1995, 1997; Povey, 1997). Most of these measurements were performed using traditional ultrasonic devices based on the pulse technique (Javanaud, 1988; McClements, 1997). This technique has been used previously in studies of the fat content of milk and creams (Miles et al. 1990), the solid-non-fat and milk fat content of fluid milk (Winder et al. 1961) and the rennet coagulation of milk (Swiatek & Kuczera, 1962; Everson & Winder, 1968; Benguigui et al. 1994; Gunesekaran & Ay, 1994; Cosgrove et al. 1996; Benedito et al. 2002; Ay & Gunasekaran, 2003). In spite of their wide use, traditional ultrasonic instruments have limited resolution; resolution of ultrasonic velocity is at best 0·1 m/sec, which does not allow the user to resolve gelation processes.

High-resolution ultrasonic spectroscopy introduced recently enables ultrasonic velocity measurements with higher resolution down to 0·2 mm/sec (Buckin & Smyth, 1999; Buckin & O'Driscoll, 2002). This resolution allows resolving details of gelation processes. The technique has been successfully applied in the ultrasonic analysis of heat-induced coagulation in calcium-fortified milk (Smyth et al. 1999), monitoring of gelation processes and enzymatic activities (Smyth et al. 2000; Buckin et al. 2002), determination of the elasticity and rheological properties of gels (Smyth et al. 2001), characterisation of biopolymers (Morrissey et al. 1999) and other systems.

In the present work, we used high-resolution ultrasonic spectroscopy to analyse rennet-induced changes in milk. Ultrasonic velocity and ultrasonic attenuation were measured in renneted milk as a function of time. The changes in these parameters over the renneting process

https://doi.org/10.1017/S0022029905001020 Published online by Cambridge University Press

were compared with results from near infra-red transmission and dynamic rheology measurements in order to fully evaluate the complex pre-gelation and gelation processes, which occur in renneted milk.

Materials and Methods

Milk sample preparation

Skim milk was prepared using an extra low heat skim milk powder (whey protein nitrogen index 7·5 mg/g powder, 90 g/kg reconstitution level). Composition of the protein after reconstitution was 3·4% of which 78% was casein.

Teagasc, Dairy Products Research Centre, Moorepark, Fermoy, Co. Cork) and MilliQ water (obtained from Millipore Super-Q system, UK). A neutral preservative (sodium azide, 0·4 g/kg) was added to the milk and it was then refrigerated at 4 °C overnight under continuous mechanical stirring.

Rennet solution

A solution containing 1 g rennet/kg prepared from rennet (Type I from calf stomach, Sigma-Aldrich Chemie Gmbh, Stenheim, Germany) and MilliQ water was used for the measurements. The rennet solution was added to the milk (5 g rennet solution/kg milk) using a pipette and agitated before filling into the sample compartments of the measuring devices.

Ultrasonic measurements

Ultrasonic measurements were carried out on renneted milk at 30 °C using the HR-US 102 high-resolution ultrasonic spectrometer (Ultrasonic Scientific, Ireland). The HR-US 102 high-resolution ultrasonic spectrometer with two 1 ml cells for ultrasonic analysis of liquid samples provides resolution of 0·2 mm/s for ultrasonic velocity and 0·2% for ultrasonic attenuation. The temperature of the sample was maintained with stability ±0·01 °C. This provided stable readings of ultrasonic velocity in differential measuring regime in water during 20 h (period of the measurements in renneted milks) of ±0·2 mm/s and 0·05% of attenuation. This stability far exceeds the required resolution, in the current research, in the measurements of ultrasonic parameters. Degassed MilliQ water was used as the calibration standard (Del Grosso & Mader, 1972). A 1 ml renneted milk sample was pipetted into the sample cell (at time 0 min) and a 1 ml untreated milk sample had previously been pipetted into the reference cell. The measuring cells were sealed with epoxy plastic caps, which prevented evaporation from the milk samples during the measurements. Ultrasonic measurements were performed in kinetic measuring regime of the HR-US 102 high-resolution ultrasonic spectrometer, which simultaneously collected sample data (velocity and attenuation) measured at several pre-selected frequencies between 2 and 15 MHz. Ultrasonic measurements were repeated several times to ensure

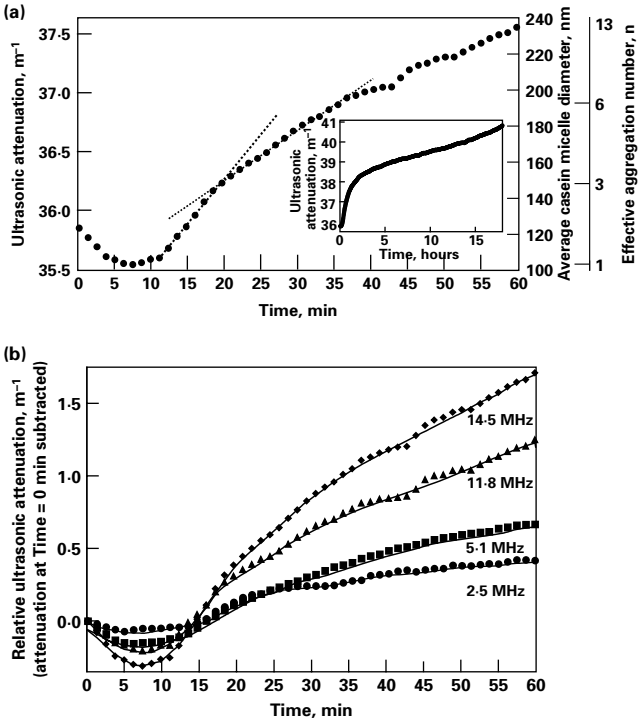


Fig. 2. (a) Ultrasonic attenuation measured at 14·5 MHz in milk during the renneting process at 30 °C using the HR-US 102 high-resolution ultrasonic spectrometer. (b) Frequency-dependence of ultrasonic attenuation measured in renneted milk at 30 °C using HR-US 102 high-resolution ultrasonic spectrometer. Calculated attenuation for each frequency shown as solid line.

reproducibility of the renneting profile. The difference in the renneting profiles obtained did not exceed the normal level of scattering of the data as shown in Figs 2 and 3.

Ultrasonic particle sizing

Calculations of the average particle size as well as scattering contributions to ultrasonic velocity and attenuation were done using Particle Size module 'Psize289' of HR-US 102 software. The software utilizes multiple scattering theories described earlier (Waterman & Truell, 1961; Allegra & Hawley, 1972; Povey, 1997). The software allows calculation of average particle size from the measured value of ultrasonic attenuation and thermophysical parameters of the continuous phase and the medium. It also allows calculations of scattering contribution to ultrasonic velocity and attenuation from a given particle size. The milk was considered as the serum continuous phase and dispersed spherical casein micelles with a volume fraction of 0·1. The thermophysical parameters of the milk serum and casein particles required for calculations; density, ultrasonic velocity, ultrasonic attenuation, viscosity, thermal conductivity, thermal expansion, and specific heat capacity, were described earlier (Kudryashov et al. 2000). As these parameters were given at 20 °C, we have

https://doi.org/10.1017/S0022029905001020 Published online by Cambridge University Press

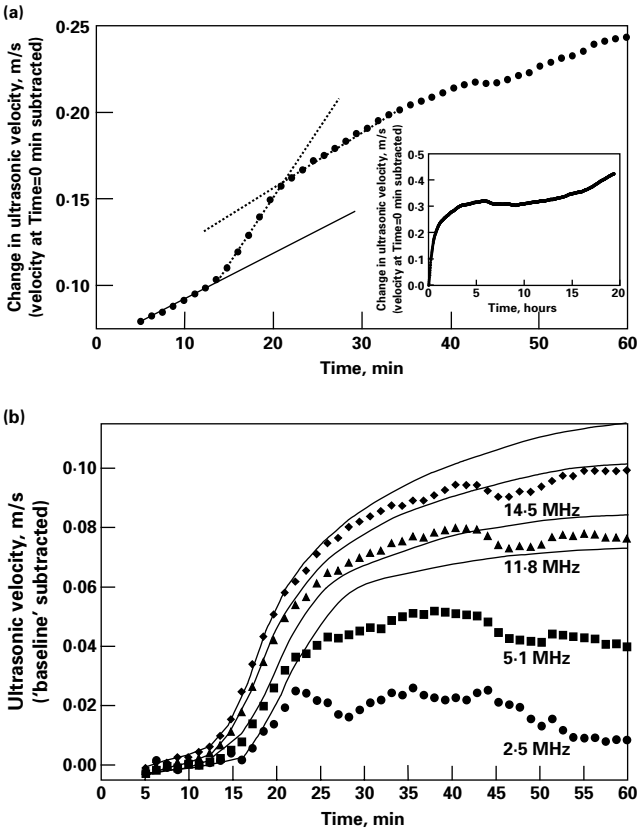


Fig. 3. (a) Change in ultrasonic velocity measured at 14·5 MHz in milk at 30 °C during the renneting process using HR-US 102 high-resolution ultrasonic spectrometer (baseline subtracted in Fig. 3b shown as solid line). (b) Frequency-dependence of ultrasonic velocity measured at 30 °C in renneted milk using HR-US 102 ultrasonic spectrometer (baseline subtracted). Predicted (structural contribution) evolution of velocity for each frequency shown as solid line.

corrected them by introducing new values for velocity in serum (1535 m/s) and attenuation divided per square of frequency ($1{\cdot}3 \times 10^{-13}$ m^{-1} s^2) in serum measured by us at 30 °C. We found that temperature dependence of the other parameters within the 10 °C difference does not have a significant effect on the particle size calculations in our system, especially on the change in the particle size during renneting.

Dynamic rheology measurements

Controlled strain rheological measurements were carried out on renneted milk at 30 °C using a PC-controlled Carrimed CSL^2 rheometer (TA Instruments, Leatherhead, UK) in controlled strain mode. The temperature was controlled by a circulating water bath. Couette geometry was used, with an outer diameter of 50 mm and an inner diameter of 48 mm. The couette was pre-set to the renneting temperature, 30 °C. Samples (20 ml) of inoculated milk (see above) were poured into the couette immediately post-inoculation. Gelation was monitored using a strain of 1% and a frequency of 1 Hz. There are discussions in literature, which suggest that under certain conditions this value of strain may affect the structure of the network of the casein particle gels, which is supported by weak interparticle interactions, especially at the beginning stages of gel formation, which can lead to a delay in the beginning of gelation observed in rheological measurements relative to ultrasonic spectroscopy (Buckin & Kudryashov, 2001; Kudryashov et al. 2001). However, this possibility does not affect the conclusions drawn in this paper.

Near infra-red (NIR) transmission measurements

Coagulation in renneted milk at 30 °C was monitored using NIR transmission measurements. The Gelograph NT (Gel Instruments AG, CH-8800 THALWIL, Switzerland) is an NIR instrument (wavelength of operation, 850 nm), which was designed for the automatic measurement of coagulation in milk in the manufacture of cheese and yoghurt. The photometric coagulation measurement measures the relative transmission of light in the NIR range (pathlength 5 mm), which is passed through a coagulating milk sample. A photodiode detects the transmitted signal and the relative transmission is evaluated electronically. The relative transmission decreases over the coagulation process due to increased absorption and scattering of the NIR light as the milk structure changes. In these measurements, a 2 ml renneted milk sample (see above) was pipetted into the Gelograph probe (NIR transmittance cell). Immersing the probe in a circulating water bath controlled the temperature. The NIR light was passed through the milk sample, the photodiode detected the transmitted signal and the decrease in relative transmission over the coagulation period was recorded.

Results

Changes in ultrasonic attenuation during the rennet-induced coagulation of milk

Figure 2a shows ultrasonic attenuation renneting profile measured at 14·5 MHz in milk at 30 °C. It was noted (Insert, Fig. 2a) that the attenuation changed continually over several hours. Attenuation measured in the first hour (Fig. 2a) underwent two major changes. Initially, attenuation decreased between 0 and 7·5 min and from 7·5 min onwards, attenuation increased. In addition to these two major changes, there were alterations in the slope of the attenuation curve at approximately 18 and 36 min (Fig. 2a). Figure 2a shows the change in the average casein micelle diameter over the first hour of the renneting process calculated using the particle sizing software module and the physical parameters of the casein particles and the continuous medium provided with the HR-US 102 software. The attenuation profile for frequency 14·5 MHz was used for the calculations. Figure 2b shows the attenuation

https://doi.org/10.1017/S0022029905001020 Published online by Cambridge University Press

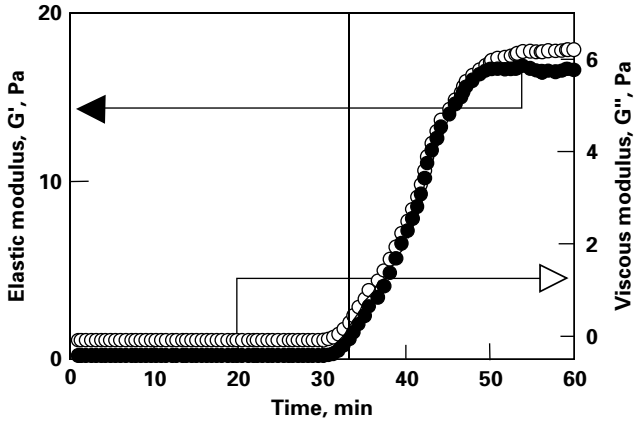


Fig. 4. Dynamic rheology measurements of renneted milk at 30 °C using Carrimed CSL^2 rheometer in controlled strain mode.

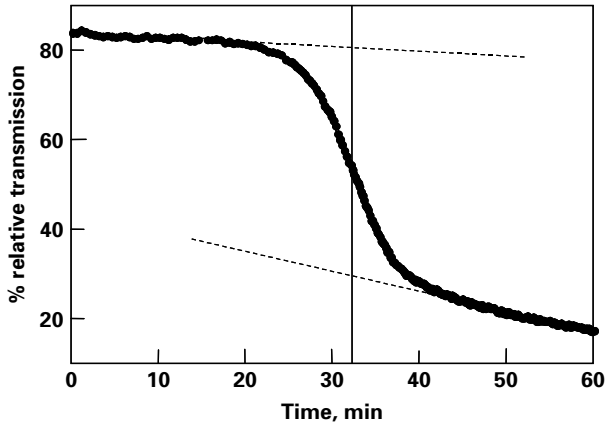


Fig. 5. Near infra-red transmission measurements of renneted milk at 30 °C using Gelograph NT.

profiles measured at several frequencies (attenuation measured at time 0 min subtracted). The higher frequencies show a higher magnitude of the initial decrease and the subsequent increase in attenuation. Figure 2b also shows the predicted ultrasonic attenuation, calculated for the four frequencies, from the particle size curve described above using the particle sizing software module of the HR-US 102.

Ultrasonic velocity changes during the rennet-induced coagulation of milk

The insert in Fig. 3a shows the change in ultrasonic velocity measured at 14·5 MHz in renneted milk over several hours, at 30 °C. Several stages of the renneting process can be clearly resolved within the first hour of the process. It must be noted that the first three measuring points have been removed from Fig. 3a, as temperature equilibration may not have been achieved at this time. Ultrasonic velocity is particularly temperature-sensitive and it has been determined from previous measurements that approximately three minutes were required for temperature equilibration of the milk sample after cell filling; ultrasonic attenuation does not have similar temperature sensitivity. The slope of the velocity curve exhibited changes at approximately 15, 20 and 40 min. Figure 3b shows the predicted ultrasonic velocity calculated from the particle size measurements described above using the particle sizing software module of the HR-US 102. These calculations only take into account the change in ultrasonic velocity caused by scattering, and do not include possible hydration and other contributions to ultrasonic velocity.

Evolution of the viscoelastic parameters in renneted milk

Figure 4 illustrates the evolution of the storage, G′, and the loss, G″, moduli in the renneted milk at 30 °C. The storage and loss moduli began increasing at approximately 30 min after addition of the rennet solution to the milk sample and levelled off at approximately 50 min. The gelation point of the renneted milk was taken as the time point when G′ reached 0·5 Pa and was determined to be at approximately 32 min after addition of the rennet solution to the milk (Fig. 4).

NIR transmission measurements in renneted milk

The results from NIR transmission measurements in renneted milk at 30 °C are shown in Fig. 5. The percent relative NIR transmission began to deviate from the baseline after approximately 20 min after the addition of rennet, decreased rapidly and then levelled off after approximately 40 min. The midpoint of this transition occurs after approximately 32 min after addition of rennet of milk.

Discussion

Changes in ultrasonic attenuation during the renneting process

Figure 2a shows the evolution of ultrasonic attenuation in renneting of milk at 30 °C. The overall attenuation in a non-homogenous colloidal dispersion is determined by intrinsic absorption of the sample components and scattering of the ultrasound wave on particles. It can be suggested that the absorption contribution to attenuation from the casein micelles and continuous phase does not change significantly during the renneting process, assuming there is little change in the internal core of the micelles. Therefore, the main contribution to the dynamic changes in attenuation observed over the renneting process can be expected to be from changes in the scattering of the ultrasound wave on the casein micelles as their size changes and aggregates are formed (see Fig. 2a).

First stage of renneting, removal of 'hairy' layer

Our data show an initial decrease in the average diameter of casein micelles from 122 nm to 102 nm in the time

https://doi.org/10.1017/S0022029905001020 Published online by Cambridge University Press

interval 0 to 7·5 min. This result is in a good agreement with the previous studies, which suggested that at the early stages of the renneting process, the hydrodynamic diameter of the micelles decreases by around 10 nm (Dalgleish, 1993). Due to the extensive hydration of the surface layer of the micelles, the true decrease in the diameter is suggested to be as high as 24 nm (Holt & Dalgleish, 1986). This is confirmed by our measurements. The decrease in micellar diameter is consistent with the loss of the 'hairy' macropeptide surface of the micelles.

Second stage of renneting, aggregation of micelles

The increase in the attenuation in the time interval between 7 and 20 min could be attributed to the aggregation of the casein micelles (Green et al. 1978; Dalgleish, 1993; Lucey, 2001). According to our results this process is accompanied by an increase of the average diameter from 102 to 145 nm. This increase corresponds to an effective aggregation number of about three micelles (see discussion below).

Third stage of renneting, formation of gel structures

At approximately 20 min, the slope of the attenuation curve and velocity curve decreases. At the same time the NIR transmission curve shows a decrease (deviation from the straight line on Fig. 5). This could be attributed to the beginning of the formation of the network from gel precursors, where the aggregates of micelles form small patches/particles of gel. This leads to the observed increase of NIR light scattered (Fig. 5), as the size of the particles became comparable with the light wavelength (850 nm).

The average particle size scale in the Fig. 2a can be recalculated into the effective aggregation number, n, using the equation: $n=(d/d_o)^3$, where d is the measured average diameter of the micellar aggregate and d_o is the diameter of the individual hairy-layer cleaved micelle (102 nm). This number represents the number of micelles in an equivalent (same volume fraction) compact aggregate. This scale is given in Fig. 2a.

Our rheological data show the beginning of the formation of 3-d network at about 30 min. At this point small patches/particles of gel begin to connect into a continuous gel network, which fills the whole sample. This time point corresponds to an effective aggregation number of six. This number was calculated on the basis of a compact spherical aggregate being formed by several micelles. However, in reality at this stage the micelles form a non-compact 3-dimensional network for which the scattering mechanism could be different from that of the compact aggregates. In this case, the non-compact network could be composed of a significantly higher number of micelles.

Post-gelation evolution of gel network

At approximately 45 min, the rheological curves levelled off, while the ultrasonic attenuation continued to increase. The end of the formation of the macroscopic gel network, which the rheological measurements are sensitive to, could explain this. However, ultrasonic attenuation shows that the microscopic structure continued to undergo some rearrangements.

Frequency-dependence of ultrasonic attenuation

Figure 2b shows the comparison of measured ultrasonic attenuation renneting profiles at different frequencies with the calculated curves. The calculations were made using the particle size curve described above using the particle sizing software module of the HR-US 102. As the particle size curve was generated from the data at one frequency (14·5 MHz), the calculated curves at other frequencies are an effective prediction of the frequency-dependence of attenuation based on the ECAH scattering theory (Epstein & Carhart, 1953; Allegra & Hawley, 1972). As can be seen from Fig. 2b, this prediction works remarkably well as the calculated attenuation is in good agreement with the experimental results.

Changes in ultrasonic velocity over the renneting process

The evolution of ultrasonic velocity in renneting milk is shown in Figs 3a and b. Ultrasonic velocity is determined by density and compressibility (or the longitudinal storage modulus), where compressibility is the major contributor to the changes in ultrasonic velocity in aggregation and similar processes (Smyth et al. 2001). There are two contributions to the compressibility of suspensions and emulsions: a structural contribution, caused by scattering of the ultrasonic waves and a contribution from intermolecular interactions, which includes intrinsic compressibility of particles and aggregates and compressibility of hydration (solvation) shells of atomic groups of solute molecules and the bulk solvent (Buckin & Smyth, 1999). Compressibility is extremely sensitive to the molecular structure and intermolecular interactions in liquids; for example, compressibility of hydrated water (water near charged ions or atomic groups of macromolecules) is different from that of bulk water by 50–100% (Buckin et al. 1989).

First stage of renneting, removal of 'hairy' layer

Figure 3a shows an initial considerable increase in ultrasonic velocity up to 15 min in the renneted milk. This could be attributed to the cleavage of peptide bonds of κ-casein and removal of the 'hairy' layer of micelles. Similar effects were observed previously in other biochemical reactions (Kudryashov et al. 2003). Cleavage of peptide bonds increases the hydration of atomic groups of amino acids of κ-casein (increase of number of atomic groups in contact with water). This leads to a decrease in compressibility and a corresponding increase in velocity, as the compressibility of atomic groups in hydration shell of the

https://doi.org/10.1017/S0022029905001020 Published online by Cambridge University Press

released groups is lower than the compressibility of bulk water (Buckin & Smyth, 1999).

Second stage of renneting, aggregation of micelles

There is an additional rise in ultrasonic velocity between 12 and 20 min, which could be attributed to the aggregation of casein micelles, which leads to a structural contribution to compressibility and ultrasonic velocity caused by scattering of the ultrasonic waves. To differentiate these structural effects from the contribution of cleavage of peptide bonds, we have subtracted the 'baseline' generated from the initial (first 10 min) slope of the velocity dependence. Figure 3b shows the evolution of ultrasonic velocity at various frequencies after subtraction of the 'baseline'. On the same Fig. (3b), the predicted curves for structural contribution to velocity are shown. These curves were calculated from the particle size curve given in Fig. 2a using the particle size software module of the HR-US 102 spectrometer. As can be seen from Fig. 3b, there is a good agreement between the predicted and experimental data in the time interval up to 20 min. This result shows that the additional increase of ultrasonic velocity at the aggregation stage can well be described by the structural contribution to ultrasonic velocity caused by the scattering of ultrasonic waves on the aggregates.

Third stage of renneting, formation of gel structures

Between approximately 20 and 40 min, the deviation of experimental points from the predicted curve is observed (Fig. 3b). This deviation is frequency-dependent and therefore cannot be explained by the contribution to ultrasonic velocity from the cleavage of peptide bonds or by intrinsic compressibility of the micelles. We can speculate that the propagation of ultrasonic waves in a gel structure can be different from that in a particle dispersion and therefore a scattering theory for dispersions may not describe the values of velocity in gel.

Post-gelation evolution of gel network

The evolution of ultrasonic velocity (similar to the attenuation profile) continues to change after 45 min, at which time the rheological curves level off. The explanation for this is as described previously for the attenuation measurements.

High-resolution ultrasonic spectroscopy is a powerful tool for analysis of microscopic processes in the formation of the milk gel. It allows the characterisation of the pre-gelation processes, which include hydrolysis and aggregation as well as at the beginning stages in the formation of the gel network. It is also sensitive to the microscopic changes in the gel at the post-gelation stage. Overall, it has good potential as a non-destructive technique to monitor renneting processes in cheese production.

We would like to thank Colette Fagan and Dr Colm O'Donnell, Department of Agricultural and Food Engineering, University College Dublin, Ireland for their assistance and support with the dynamic rheology and NIR transmission measurements. The Strategic Grant Scheme ST/2000/046 from Enterprise Ireland and Grant 00R&DTD59, 2001 from the Department of Agriculture, Food and Rural Development, supported this work.

References

Allegra JR & Hawley SA 1972 Attenuation of sound in suspensions and emulsions: Theory and Experiments. *Journal of Acoustical Society of America* **51** (5, Part 2)1545–1564

Ay C & Gunasekaran S 2003 Numerical method for determining ultrasonic wave diffusivity through coagulating milk system. *Journal of Food Engineering* **58** 103–110

Benedito J, Carcel JA, Gonzalez R & Mulet A 2002 Application of low intensity ultrasonics to cheese manufacturing processes. *Ultrasonics* **40** 19–23

Benguigui L, Emery J, Durand D & Busnel JP 1994 Ultrasonic study of milk clotting. *Lait* **74** 197–206

Buckin VA, Kankiya BI, Bulichov NV, Lebedev AV, Gukovsky IY, Chuprina VP, Sarvazyan AP & Williams AR 1989 Measurement of anomalously high hydration of (dA)n × (dT)n double helices in dilute solution. *Nature* **340** 321–325

Buckin V & Kudryashov E 2001 Ultrasonic shear wave rheology of weak particle gels. *Advances in Colloid and Interface Science*, **89–90** 401–422

Buckin V, Kudryashov E & O'Driscoll B 2002 High-resolution ultrasonic spectroscopy for material analysis. *American Laboratory* (*Spectroscopy Perspectives Supplement*), March, 28 30–31

Buckin V & O'Driscoll B 2002 Expanding laboratory applications for ultrasonic spectroscopy. *Laboratory Equipment*, June 15–18

Buckin V & O'Driscoll B 2002 Ultrasonic waves and material analysis: Recent advances and future trends. *Lab Plus International* **16** (3) 17–21

Buckin V & Smyth C 1999 High-resolution ultrasonic resonator measurements for analysis of liquids. *Seminars in Food Analysis* **4** (2) 113–130

Cosgrove N, O'Donnell C, Li W & O'Callaghan D 1996 Development of an ultrasonic instrumentation system to monitor gel development during enzymatic coagulation of milk. *Irish Journal of Agricultural and Food Research* **35** (2) 221–222

Dalgleish DG 1979 Proteolysis and aggregation of casein micelles treated with immobilized or soluble chymosin. *Journal of Dairy Research* **46** 653–661

Dalgleish DG 1993 The enzymatic coagulation of milk. In *Cheese: Chemistry, Physics and Microbiology*. pp. 1–36. Vol. 1, 2nd Edn. (Ed. PF Fox) London: Chapman & Hall

Del Grosso VA & Mader CW 1972 Speed of sound in pure water. *Journal of the Acoustical Society of America*, **52** (5) 1442–1446

Epstein PS & Carhart RR 1953 The absorption of sound in suspensions and emulsions. I. Water fog in air. *Journal of the Acoustical Society of America* **25** 553–560

Everson TC & Winder WC 1968 Rennet coagulation test with a recorded output. *Journal of Dairy Science* **51** 940–947

Fox PF & Sweeney PLH 1998 Chemistry and biochemistry of cheese and fermented milks. In *Dairy Chemistry and Biochemistry* pp. 379–436. London: Blackie Academic & Professional

Green ML, Hobbs DG, Morant SV & Hill VA 1978 Intermicellar relationships in rennet-treated separated milk. II Process of gel assembly. *Journal of Dairy Research* **45** 413–422

Gunasekaran S & Ay C 1994 Evaluating milk coagulation with ultrasonics. *Transactions of the ASAE* **37** 857–862

Holt C & Dalgleish DG 1986 Electrophoretic and hydrodynamic properties of bovine casein micelles interpreted in terms of particles with an outer hairy layer. *Journal of Colloid and Interface Science* **114** (2) 513–524

Javanaud C 1988 Applications of ultrasound to food systems. *Ultrasonics* **26** 117–123

Kudryashov E, Hunt NT, Arikainen EO & Buckin V 2001 Monitoring of acidified milk gel formation by ultrasonic shear wave measurements. High-frequency viscoelastic moduli of milk and acidified milk gel. *Journal of Dairy Science* **84** 375–388

Kudryashov E, Smyth C, Duffy G & Buckin V 2000 Ultrasonic high-resolution longitudinal and shear wave measurements in food colloids: monitoring of gelation processes and detection of pathogens. *Progress in Colloid and Polymer Science* **115** 287–294

Kudryashov E, Smyth C, O'Driscoll B & Buckin V 2003 Analysing chemical reactions in real time with ultrasonic spectroscopy. *Spectroscopy* October 2003 **18** (10) 26–32

Lucey JA 2001 Formation and physical properties of milk protein gels. *ADSA Foundation Scholar Award* 1–31

McClements DJ 1995 Advances in application of ultrasound in food analysis and processing. *Trends in Food Science and Technology* **6** 293–299

McClements DJ 1997 Ultrasonic characterization of foods and drinks: Principles, methods, and applications. *Critical Reviews in Food Science and Nutrition* **37** (1) 1–46

McClements DJ, Povey M & Dickinson E 1993 Absorption and velocity dispersion due to crystallization and melting of emulsion droplets. *Ultrasonics* **31** 433–437

Miles CA, Shore D & Langley KR 1990 Attenuation of ultrasound in milks and creams. *Ultrasonics* **28** 394–400

Morrissey S, Kudryashov E & Buckin V 1999 Surfactant-DNA complexes in low ionic strength dilute solutions. *Progress in Colloid and Polymer Science* **112** 71–75

Payens TAJ 1977 On enzymatic clotting processes II. The colloidal instability of chymosin-treated casein micelles. *Biophysical Biochemistry* **6** (3) 263–270

Povey MJW 1997 *Ultrasonic Techniques for Fluids Characterization*. London: Academic Press Inc

Smyth C, Dawson K & Buckin VA 1999 Ultrasonic analysis of heat-induced coagulation in calcium-fortified milk. *Progress in Colloid and Polymer Science* **115** 287–294

Smyth C, Kudryashov E & Buckin V 2001 High-frequency shear and volume viscoelastic moduli of casein particle gel. *Colloids and Surfaces* **183** 517–526

Smyth C, Kudryashov E, Duffy G & Buckin VA 2000 Ultrasonic high-resolution longitudinal and shear wave measurements in food colloids: Monitoring of gelation processes and detection of pathogens. *Progress in Colloid and Polymer Science* **115** 287–294

Swiatek A & Kuczera S 1962 Studies on the changes of the mechanical properties of milk during renneting. *XVI International Dairy Congress I* p D550, D562–D568

Waterman PC & Truell R 1961 Multiple scattering of waves. *Journal of Mathematical Physics* **2** 512–537

Winder WC, Conigny NP & Rodriguez-Lopez B 1961 An ultrasonic method for the measurement of solids non-fat and milk fat in fluid milk, II. An evaluation of the method. *Journal of Dairy Science* **44** 1165–1169