

Ultrasonic study of milk clotting

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Summary — The aim of this study was to understand the structure-properties relationships of the different physical gels built by milk clotting. The investigation of their clotting mechanisms and an approach of the mechanical properties of gels were realized. Ultrasonic wave propagation, a non-destructive investigation tool, was used as an original and privileged means of investigation for milk clotting study. The work presented in this article shows the possibility to obtain an ultrasonic signal related to the different steps of milk clotting and to some of their key parameters. In addition, two distinct ultrasonic response curves have been obtained for rennet and acid coagulation. From the obtained results, it appears to be possible to use ultrasonic techniques for monitoring milk coagulation, however, more research is needed for their validation as a routine tool.

milk gel / ultrasonic wave / physical gel / rennet milk gel / acid milk gel / casein micelle

Résumé — *Étude ultrasonore de la coagulation du lait.* Cette étude avait pour principal objectif la compréhension des relations structure-propriétés des gels physiques engendrés par coagulation du lait. Cet objectif nécessitait, d'une part, la connaissance des mécanismes de coagulation, et d'autre part, la détermination des propriétés mécaniques du système. La propagation des ondes ultrasonores, compte tenu de son caractère non destructeur vis-à-vis du système étudié, constitue un moyen d'investigation original et de choix pour l'exploration des phénomènes de coagulation du lait. Les travaux présentés dans cet article montrent qu'il est possible d'obtenir un signal ultrasonore sensible aux différentes étapes de coagulation du lait, ainsi qu'à certains facteurs de variation déjà connus. De plus, aux 2 types de coagulations étudiés, coagulation présure et acide, correspondent 2 réponses ultrasonores distinctes. Les résultats présentés permettent a priori d'envisager de nouvelles perspectives quant aux applications des techniques ultrasonores dans le domaine de la coagulation du lait ; il est à noter cependant que leur validation en tant qu'outil de contrôle reste à faire.

lait / onde ultrasonore / gel physique / coagulum présure / coagulum acide / micelle de caséines

INTRODUCTION

Physical gels, from enzymatic or acid milk coagulations (Dalglish, 1982; Heertje *et al*, 1985) are very weak; the different techniques of investigation which aim to study the aggregate formation and mechanical properties of gels need to take into account this weakness.

The ultrasonic wave propagation techniques, easy to use and propagate in the system are non-destructive, and so, particularly adapted to follow those gelation processes (Emery, 1989). The main characteristics of a developing network are its elastic properties which govern the wave propagation parameters.

The kinetics of the different types of coagulations, the influence of various experimental conditions (temperature, pH, etc), the mechanical properties of viscoelastic milk gels and the network development are the main targets of this study performed on a milk model in order to avoid the problems of experiment reproducibility.

The milk world is very complex (Schmidt, 1982), so our investigations on milk clotting are related and discussed with respect to the different classical models of milk clotting.

MATERIALS AND METHODS

Reconstituted skim milk preparation

In most of our experiments, a skim milk powder was used, kindly provided by Daniel Carasso International Research Center (BSN, Gervais-Danone, Le Plessis Robinson, France); indeed, the variability of fresh milk (due to season, breed, lactation stage, etc) requires the use of a constant milk model.

12 g of skim milk powder were added to 88 g of bidistilled water heated at 60°C. This preparation was strongly mixed during 30 min at room temperature. This solution was then ready to be renneted or treated by microorganisms.

For enzymatic gelations, the rennet used was provided by Boll society (Arpajon, France); clotting activity was 0.1 g of chymosin for 1000 g of milk and the ratio rennet-reconstituted milk was 0.1%. The rennet was kept at 4°C and the dilution, before start of the experiment, was made for half a day.

For acid gelations, 1 ml of starter culture ('DVS ST20' Boll Society stocked at -18°C for less than 2 months) was added at 10 ml of model milk. The required amount of rennet or microorganisms, at the chosen temperature, was added in the cell. A gentle stirring was applied in order to avoid bubble formation (big problem for ultrasonic wave propagation). 2 or 3 min are needed to reach the study temperature. In order to have conditions comparable to those in the dairy factory, the preparation did not contain any antibacterial products (thiomersal, sodium azide, etc).

Ultrasonic propagation in liquids

Two types of mechanical waves propagate in condensed matter (Papadakis, 1976): shear and longitudinal waves. In the case of aqueous solutions, shear waves are strongly damped, so only longitudinal waves can be used.

The propagation of longitudinal waves, in the direction x , is governed by the following equation:

$$d^2u/dt^2 = (M^*/\rho) \cdot (d^2u/dx^2) \quad (1)$$

where u is the displacement in the direction x , ρ the mass density and M^* the complex longitudinal modulus which results from a pure dilatation (compression) modulus K^* and a pure shear modulus G^* according to:

$$M^* = K^* + 4/3 G^* \quad (2)$$

For an harmonic longitudinal excitation (longitudinal transducers) in an infinite medium, the solution is harmonic and at the frequency $f = \omega/2\pi$:

$$u(x,t) = u_0 \cdot e^{-\alpha x} \cdot e^{i\omega(t-x/V)} \quad (3)$$

The real M' and imaginary M'' parts of the modulus M^* can be determined by the measurement of absorption coefficient α and longitudinal velocity V of the wave: if $\alpha V/\omega \gg 1$:

$$M' \sim \rho \cdot V^2 \tag{4}$$

$$M'' \sim 2\rho \cdot V^3 \cdot \alpha/\omega \tag{5}$$

M^* can be frequency-dependent due to molecular interactions in the medium; two kinds of relaxation processes can be involved: bulk and shear. In the case of reconstituted milk, and in the high frequency range investigated (2–100 MHz), the bulk modulus can be considered as already relaxed, thus only a shear relaxation process of relaxation time τ would be concerned. If the relaxation is distributed according to a distribution function $h(\tau)$ then: (6) (7)

$$M' = K_\infty + A \int_0^\infty \frac{\omega^2 \tau^2}{1 + \omega^2 \tau^2} h(\tau) d\tau \tag{6}$$

$$M'' = A \int_0^\infty \frac{\omega \tau}{1 + \omega^2 \tau^2} h(\tau) d\tau \tag{7}$$

where $A = (K_\infty - K_0)$ is the amplitude of the process and K_∞, K_0 are the values of K at infinite and zero frequency respectively.

Experimental device

Various methods for making ultrasonic velocity and absorption measurements have been developed over the years. Most of the advantages and disadvantages of each method have been reviewed by Papadakis (1976). In our experiments, we used a completely automated method of measuring attenuation and velocity using quadrature phase detection (fig 1) of Matec Instruments' MBS system (MBS 8000, Boston, USA); methods for measuring ultrasonic velocity using phase detection were described by Williamson (1969). Variation of amplitude, in mV, of the received signal, and the phase variation, between emitted and received signals, detected in phase and in quadrature phase, allow us to determine $\Delta\alpha$ and ΔV . The use of phase detection has two major advantages: automated measurements are much easier to achieve than the other methods and signals may be recovered from noise using simple computer averaging techniques. The method used involves interactive automatic control of the frequency and measurement of phase relationships. The time elapsed between the transmitted and received signals is related to the

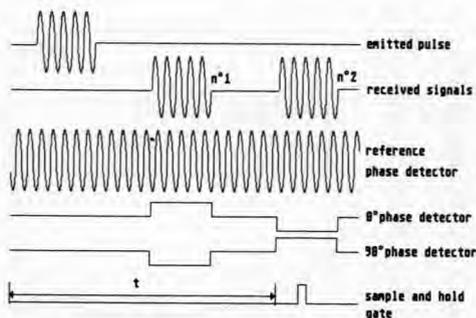


Fig 1. Ultrasonic method of measuring ultrasonic wave attenuation and velocity using quadrature phase detection.

Méthode de mesure de l'absorption et de la vitesse de l'onde ultrasonore utilisant la détection quadratique de phase.

frequency and the phase by the equation (8); the data obtained are shown in figure 1.

$$t = \Phi/2\pi f \tag{8}$$

where Φ is a phase angle much larger than 2π and f is the frequency in Hz.

To complete our ultrasonic study, a very sensitive strain controlled coaxial cylinders rheometer (Contraves Low-Shear 40) was used at the limit of its application range in order not to disturb the system.

RESULTS

Ultrasonic propagation in reconstituted milk; frequency investigation

The ultrasonic properties of a reconstituted milk were determined by fast Fourier transform ultrasonic spectroscopy in the 60–200 MHz range; they show clearly relaxation processes below 150 MHz, which might be attributed to micellar particles of milk and their interactions with the solvent (fig 2).

During coagulation, the ultrasonic absorption increased because of the increasing of

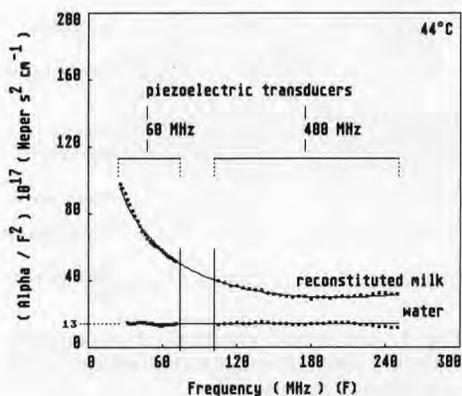


Fig 2. Ultrasonic relaxation spectrum for a reconstituted milk.

Spectre de relaxation ultrasonore d'un lait reconstitué.

the mechanical modulus of the liquid and it was followed at constant frequency with an ultrasonic burst technique (Matec MBS 8000). For a good compromise between sensitivity and easy experimental realization, a frequency of 60 MHz was chosen.

Rennet coagulation kinetics

At 37°C, and for the 'standard' conditions, as shown in figure 3, reconstituted milk clotted between 8 and 9 min; the ultrasonic absorption increased with time and the transit time decreased, indicating increasing ultrasonic velocity. The magnitude of the change in absorption is $\Delta\alpha$ (60 min, 37°C) = 32 Neper/m and of 0.47 m/s for the velocity after 1 h. These quantities do not reach a plateau-value even after 6 h, showing that the medium is still changing at that time. For lower temperatures (fig 4), the process is similar but the variations of absorption and rate are smaller (figs 5, 6): $\Delta\alpha$ (60 min, 25°C) = 21 Neper/m (at 60 MHz). In addition, different lag times are observed: the lower the temperature, the longer the lag time is (Zoon et al, 1988a, b).

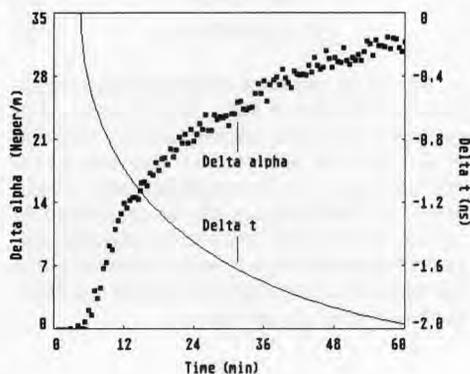


Fig 3. Gelation kinetic of renneted (1/10000, 0.1%) reconstituted (12%) milk at 37°C. Ultrasonic transmission at 60 MHz.

Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 37°C. Transmission ultrasonore à 60 MHz.

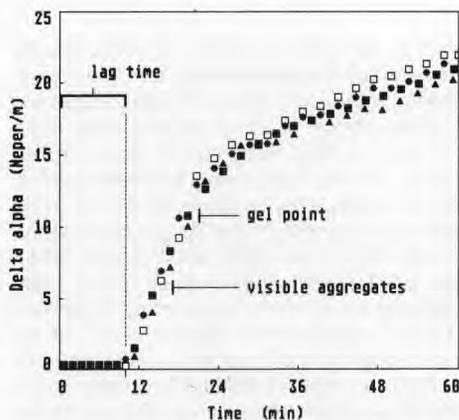


Fig 4. Gelation kinetic of renneted (1/10000, 0.1%) reconstituted (12%) milk at 25°C. Ultrasonic transmission at 60 MHz. Different symbols for each experiment.

Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 25°C. Transmission ultrasonore à 60 MHz. Différents symboles pour chaque expérience.

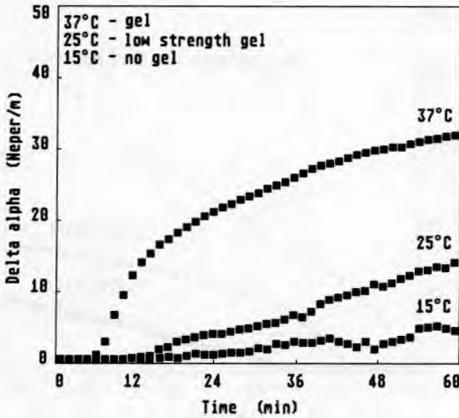


Fig 5. Enzymatic gelation kinetic of renneted (1/10000, 0.1%) reconstituted (12%) milk at 15, 25 and 37°C. Ultrasonic transmission at 60 MHz. Temperature influence • $\Delta\alpha = f(\text{time})$.
Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 15, 25 et 37°C. Transmission ultrasonore à 60 MHz. Influence de la température. $\Delta\alpha = f(\text{temps})$.

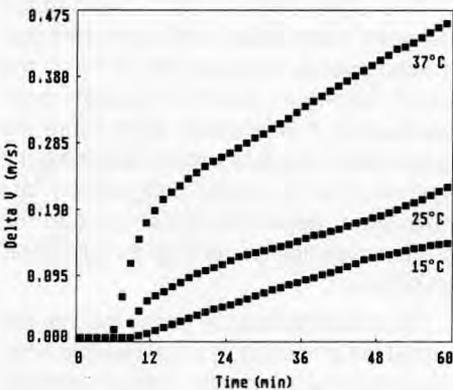


Fig 6. Enzymatic gelation kinetic of renneted (1/1000, 0.1%) reconstituted (12%) milk at 15, 25 and 37°C. Ultrasonic transmission at 60 MHz. Temperature influence $-\Delta v = f(\text{time})$.
Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 15, 25 et 37°C. Transmission ultrasonore à 60 MHz. Influence de la température. $\Delta v = f(\text{temps})$.

Acid coagulation kinetics (fig 7)

The acid coagulation was done at 44°C. The evolution of $\Delta\alpha$ with time was different from that in the rennet coagulation. For acid coagulation, a plateau-value is reached for both $\Delta\alpha(t)$ and $\Delta V(t)$. The magnitudes of both $\Delta\alpha$ and ΔV are much more larger than for the other process, respectively 100 Neper/m and 3.5 m/s. A parallel pH monitoring provides a way to understand this ultrasonic study of milk gelations.

Influence of milk powder and calcium chloride concentrations

Milk powder concentration (fig 8)

As shown in figure 8, the lag time is independent of powder concentration but, after 1 h, the $\Delta\alpha$ (60 min, 25°C) values are superior for higher concentrations: the ultrasonic parameters seem closely dependent on mechanical properties of final milk product (here an enzymatic coagulum).

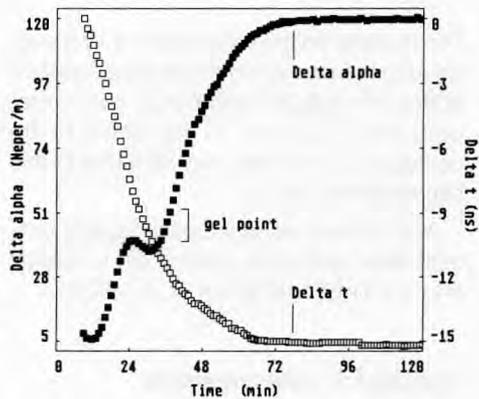


Fig 7. Acid gelation (DVS, 1/10) kinetic of reconstituted (12%) milk at 44°C. Ultrasonic transmission at 60 MHz.
Cinétique de gélification acide (ferments DVS 1/10) d'un lait reconstitué (12%) à 44°C. Transmission ultrasonore à 60 MHz.

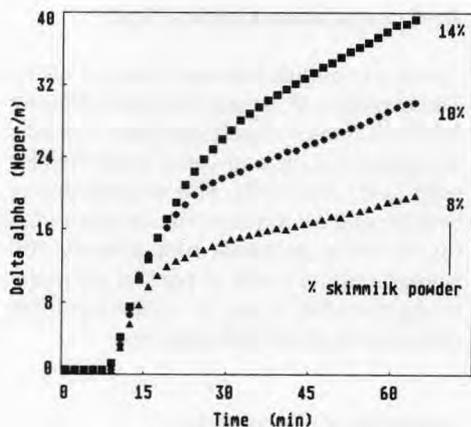


Fig 8. Enzymatic gelation kinetic of renneted (1/10000, 0.1%) reconstituted milk at 25°C. Ultrasonic transmission at 60 MHz. Influence of milk powder concentration.
Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 25°C. Transmission ultrasonore à 60 MHz. Influence de la concentration en poudre de lait.

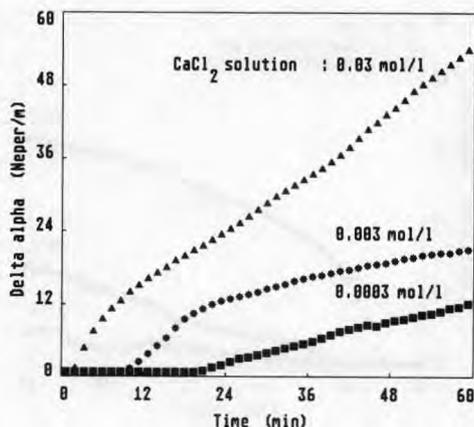


Fig 9. Enzymatic gelation kinetic of renneted (1/10000, 0.1%) reconstituted milk at 25°C. Ultrasonic transmission at 60 MHz. Influence of added calcium chloride.
Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 25°C. Transmission ultrasonore à 60 MHz. Influence d'un ajout de chlorure de calcium.

Calcium chloride concentration added (fig 9)

The lag time before coagulation is very sensitive to the calcium chloride concentration added: for a 0.03 mol/l CaCl_2 concentration, this lag time is too short to be detectable. $\Delta\alpha$ is also very sensitive to this concentration (fig 9).

It is known that an increased CaCl_2 concentration leads to a quicker gel formation and to a firmer gel (Zoon *et al*, 1988c).

Rheological measurements

The complementary study made with a very sensitive strain controlled coaxial cylinder rheometer (Contraves Low-Shear 40) allowed us to realize a rheological study with such a system.

At a shear rate of 1 s^{-1} , competition between destruction and aggregate formation seems to occur. At 10^{-3} s^{-1} , the used conditions seem absolutely non-destructive; it is possible to measure the aggregation progress without disturbing the system. During rennet coagulation, the Low-Shear apparatus is sensitive to the system evolution earlier than the ultrasonic technique.

To obtain information on the mechanical rennet gel properties, several discrete measurements were made in the harmonic mode at 0.1 Hz. Varying with the added calcium chloride concentration, the results obtained (tables I, II) through the low frequency rheological study confirm, as does the ultrasonic technique, the added calcium chloride effect mentioned in the literature (Zoon *et al*, 1988c): acceleration of the rennet coagulation, greatest strength of gel.

Table I. Enzymatic gelation kinetic of renneted (1/10000, 0.1%) reconstituted milk at 25°C. Low-Shear rheometer, discrete measurements in harmonic mode (0.1 Hz) – 0.003 mol/l CaCl₂ added. *Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 25°C. Rhéomètre Low-Shear, mesures ponctuelles en régime harmonique (0,1Hz), 0,003 mol/l de CaCl₂ ajouté.*

		Time after renneting (min)			
		15	30	45	60
0.003 mol/l	G" (Pa)	–	1.20	1.98	2.16
	G'	0.86	4.01	3.95	4.20
CaCl ₂	G	0.86	4.19	4.42	4.73

Table II. Enzymatic gelation kinetic of renneted (1/10000, 0.1%) reconstituted milk at 25°C. Low-Shear rheometer, discrete measurements in the harmonic mode (0.1 Hz) at fixed shear amplitude (30%). Various concentrations of added calcium chloride. *Cinétique de gélification présure (1/10 000, 0,1%) d'un lait reconstitué (12%) à 25°C. Rhéomètre Low-Shear, mesures ponctuelles en régime harmonique (0,1 Hz) à amplitude de cisaillement fixée (30%). Différentes concentrations en CaCl₂ ajouté.*

		Time after renneting (min)	
		15	60
0.03 mol/l CaCl ₂	G"(Pa)	1.32	2.15
	G'	3.96	4.47
	G	4.17	4.97
0.003 mol/l CaCl ₂	G"	–	2.16
	G'	0.86	4.20
	G	0.86	4.73
no CaCl ₂	G"	–	1.78
	G'	–	4.09
	G	–	4.46

For those systems, the linear viscoelastic regime is extremely limited. Even small strains can destroy the gel. The true modulus for those systems can only be measured with a high sensitivity apparatus such as

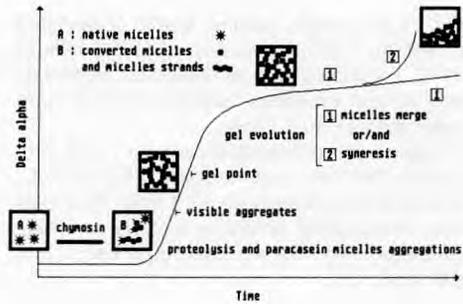


Fig 10. Enzymatic gelation kinetic of renneted reconstituted milk. Ultrasonic $\Delta\alpha = f(\text{time})$ interpretation. *Cinétique de gélification présure d'un lait reconstitué. Interprétation de la variation d'absorption ultrasonore en fonction du temps.*

the LS40. We conclude that, in this case, LS40 was not an especially suitable apparatus.

DISCUSSION

Comparison of the results of this paper and the literature (Heertje *et al*, 1985; Walstra and Van Vliet, 1986) suggests the following interpretation for both investigated milk gelation mechanisms.

In the rennet coagulation (fig 10), the layer of the flexible hairs which induces an

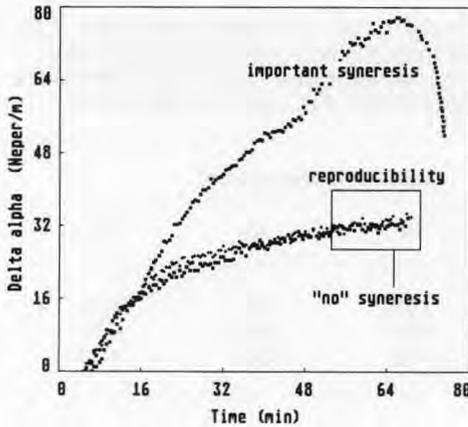


Fig 11. Enzymatic gelation kinetic of renneted (1/10000, 0.1%) reconstituted (12%) milk at 37°C. Examples with an important syneresis and without syneresis (superposition of three experiments • $\Delta\alpha = f(\text{time})$).

Cinétique de gélification présure (1/10 000, 0.1%) d'un lait reconstitué (12%) à 37°C. Transmission ultrasonore à 60 MHz. Exemples avec importante synérèse et sans synérèse (superposition de trois expériences. $\Delta\alpha = f(\text{temps})$).

'electrosteric' repulsion between the micelles is destroyed by the limited enzymatic proteolysis (chymosin attacks specifically Phe105-Met106/y residues of kappa caseins); so, first step, the hydrophilic glycomacropéptide leaves the micellar phase. Second step, after this physico-chemical modification of micelles, the aggregation starts and leads to a three-dimensional network initially composed here of strands of micelles linked through nodes resulting themselves from micelles' aggregation (Walstra and Van Vliet, 1986; Zoon *et al*, 1988a). It is important to mention that ultrasonic waves are sensitive from the beginning of the system's evolution: during the first step, proteolysis and aggregation, ultrasonic absorption and rate increase. The gel point is not visible on $\Delta\alpha$ or ΔV versus time. The ultrasonic parameters continue to increase even during the less known events

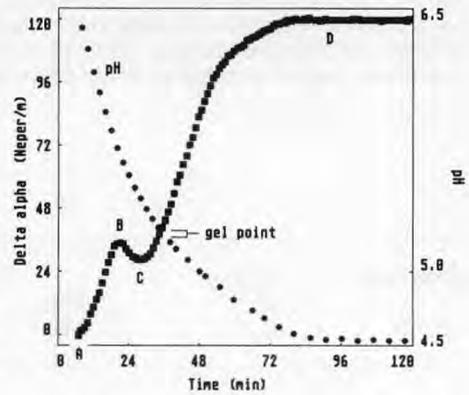


Fig 12. Acid gelation (DVS, 1/10) kinetic of reconstituted (12%) milk at 44°C. Ultrasonic (60 MHz) and pH study.

Cinétique de gélification acide (ferments DVS 1/10) d'un lait reconstitué (12%) à 44°C. Transmission ultrasonore à 60 MHz et étude en pH.

taking place after the gel point. So, ultrasonic waves are sensitive to gel evolution, the third step of rennet coagulation. The precocity of sporadic syneresis, solvent release from the gel due to the reorganization of the gel structure (Walstra *et al*, 1985) might be one of the consequences of the rapid rennet coagulation process (fig 11). Not many techniques pretend to follow practically all the rennet coagulation steps.

In acid coagulation (fig 12), resulting from progressive acidification of milk by microorganisms digesting lactose and producing lactic acid, several steps (A to D) appear. According to the literature (Heertje *et al*, 1985; Walstra and Van Vliet, 1986) acid milk gel formation appears clearly as a process more complex than a simple native micelle aggregation; the first step (A to B), between pH 6.4 and pH 5.6, corresponds to the micelle demineralization (colloidal calcium

phosphate solubilization); during this step, the $\Delta\alpha$ increases. It might be explained by a swelling of micelles (giving increased viscosity) as already observed (Payens, 1989). The second step (B to C), with a small decrease of $\Delta\alpha$ begins at the end of demineralization (near pH 5) and typical pH of caseins dissociations from micelles: B (pH 5.6) is the pH of first casein dissociations and C (pH 5.4) the maximal dissociation. That indicates clearly that ultrasonic waves are not only sensitive to particle aggregation; the solute-solvent (micelles-whey) change probably influences ultrasonic propagation. A gel point is observed near pH 5.1 and $\Delta\alpha$ increases again and finally reaches a stable plateau at pH 4.6 (D). During this step, as in rennet coagulation, ultrasonic waves are sensitive to particle aggregation and network development. One explanation for the plateau observed only in the case of acid coagulation could be the following: in acid coagulation, the micellar rearrangements could lead to a more stable configuration of the different network compounds resulting from a temporary equilibrium reached earlier (constant value of $\Delta\alpha$ and Δt ; the micelles' destructure into submicelles could generate a slower gelation process, leading to a better organization before and after the gel point. The gel formation through a rennet process would be too fast and could probably involve some later rearrangements prohibiting any equilibrium.

CONCLUSION

Ultrasonic techniques are a convenient tool to study biological networks (Emery, 1989). They allow to follow the whole process from the starting aggregation to gelation.

The ultrasonic signal development is sensitive to the classical parameters of milk gelation: temperature, milk powder concentration, added calcium chloride.

Above 40 MHz, two different ultrasonic results allow to distinguish the two main coagulation mechanisms, the enzymatic one and the acid one. The ultrasonic techniques are not only sensitive to aggregates' formation and size, but also to the involved mechanisms. In the acid coagulation, the ultrasonic experiments, made in parallel with the progressive acidification of the milk-microorganism system and compared to the zeta potential-pH study, allow to propose the following assumption: the micellar rearrangements, *ie* the exchanges between the colloidal phase and the serum, may influence the ultrasonic propagation by changing the micelle-serum interface, consequence of caseins dissociations (Walstra and Van Vliet, 1986).

The ultrasonic absorption and velocity variations correspond to the complex longitudinal modulus (M^*) development and may be mainly due to the contribution of the shear modulus G^* . It may be possible, as for covalent networks (Emery, 1989), to use the ultrasonic techniques to measure those moduli with a great accuracy and without disturbing the system.

Finally, during the gel evolution, the ultrasonic techniques may detect the syneresis phenomenon if the contraction of gel takes place inside the wave path: then, the system becomes heterogeneous.

With respect to these preliminary results, an industrial technological device should be developed and tested to validate the use of ultrasonic waves to follow on-line the texture evolution from the native milk to the elaborated milk products.

From a fundamental point of view, it would be worth to apply the ultrasonic techniques to better characterized systems such as specific caseins. Moreover, a broad frequency range is needed. Such a study, mainly concerned with a macroscopic approach of the gelation, may be achieved by a structural characterization of the first

aggregates (electron microscopy, diffusion techniques, etc).

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