2D-IR-COSS as a tool in understanding milk rennet coagulation processes

C. Giardina, N. Sinelli, T.M.P. Cattaneo and R. Giangiacomo

Istituto Sperimentale Lattiero-Caseario, via A. Lombardo, 11 – 26900 Lodi - ITALY

Introduction

Water molecules rearrangements during the process of coagulation are the main event in the passage from milk to curd. From our previous works[1-2], we knew that the major structural event taking place during the early phases of the coagulation process was water rearrangement and that the water combination band in the region around 1930 nm well described this event.

Two-dimensional infrared correlation (2D-IR-COSS) spectroscopy[3] is an analytical technique based on time-resolved detection of IR signals to study molecular interactions. The ability of this technique is to identify intra- and intermolecular interactions and to deconvolute highly overlapped IR bands by spreading them over the second spectral dimension.[4] In this work, the 2D-IR-COSS was applied to verify its ability in highlighting deviations in the coagulation process due to changes in the process variables like temperature, pH, and rennet concentration.

Materials and methods

Materials

Standard (A) rennet coagulation test was carried out in duplicate at 35°C, using reconstituted skim milk powder (RMSP) at 10% of solid content (w/w) in $CaCl_2$ 0.05% as substrate and liquid calf rennet (LCR) as clotting agent. LCR (containing 17% of bovine pepsin, strength 1:16000 Soxhlet units, Caglificio Clerici, Cadorago, CO, Italy) was diluted to 0.8% (v/v) with distilled water and added to milk at 2%.

Different coagulation tests were carried out changing the process variables one by one: temperature (B), pH (C), rennet concentration (D). Experimental variable parameters are reported in detail in Table 1.

	SUBSTRATE	LCR	T (°C)	pH
A*	RSMP	2%	35	6.5
B	RSMP	2%	30	6.5
C	RSMP	2%	35	5.6
D	RSMP	4%	35	6.5

A* = standard process

Methods

NIR spectra were collected by a dispersive spectrometer InfraAlyzer 500 (Bran+Luebbe, Norderstedt, Germany) in transflectance mode as *log (1/R)* in the range 1100-2500 nm at 4 nm intervals, using Sesame software (Bran+Luebbe, Germany). The instrument was equipped with a

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thermostatable liquid sample cell. Temperature was controlled by an external circulating bath (Haake, mod. F3-CH, Karlsbruhe, Germany). Milk samples were injected into the cell after the addition of the clotting agent, and spectra were recorded in sequence every 71 seconds until curd formation.

Data processing

Synchronous NIR/NIR correlation intensities were calculated for the spectra by using the generalised 2D method according to Noda.[5] This method enables the study of correlations resulting from fluctuations in spectral intensities, as a function of any physical variable other than time. In 2D IR, a spectrum defined by two independent wavelengths is generated by a cross-correlation analysis of dynamic fluctuations of IR signals induced by external perturbations. 2D contour maps were plotted with the use of Grams/32 AI software (Galactic Ind. Co., Salem, NH, USA).

Results and discussion

Figure 1 shows a 3D representation of the synchronous 2D NIR correlation spectrum of the standard (A) coagulation process. The value of correlation intensity is plotted as a function of two independent wavelengths. Such a plot gives an excellent visual image of 2D NIR spectra, but contour map representation is more often used in analysing 2D spectra for detailed interpretation of fine features of a complicated spectrum.

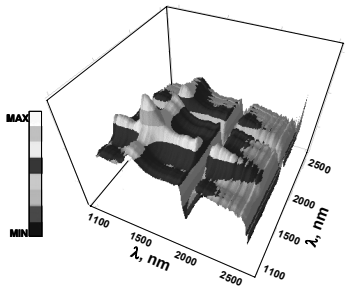

Figure 1. 3D representation of the synchronous 2D NIR correlation spectrum of the standard process (A).

From the full region, the areas of interest were isolated and their contour maps were analysed. Standard (A) rennet coagulation (Figure 2) shows auto peaks at 1928 and 1940 nm indicating that the spectral features at these positions vary in phase with each other. The appearance of positive cross-peak between the autopeaks located at 1928 and 1940 nm reveals that the changes are correlated and occur in the same direction.

These absorptions[6] above 1924 nm suggest that the process involves H-bonded water molecules in particular, rather than free molecules. When k-casein starts to release caseinomacropeptide (CMP),[7] micelles reorganise and consequently water is finding a new equilibrium, by breaking the old bonds and creating new ones. This mechanism could involve especially the water S1 form, i.e. with 1 H-bond.

Analysing the contour map relating to B (Figure 3), only one autopeak at about 1900 nm is observed.

This map is very different from that of A: 30°C is not the temperature where the enzyme can work under its optimal conditions and give its maximum contribution to the coagulation process.

Figure 4 shows the contour map relating to C in which pH value is settled at 5.6. Two prominent autopeaks are shown at 1924 and 1932 nm: their values of correlation intensity achieve the maximum as in A system. From the sign of the cross peak, it is evident that the 1924 nm band correlates positively with the one at 1932 nm. The presence of this cross peak indicates that both free (1924 nm) and bonded (1932 nm) water molecules are involved in the progress of coagulation.

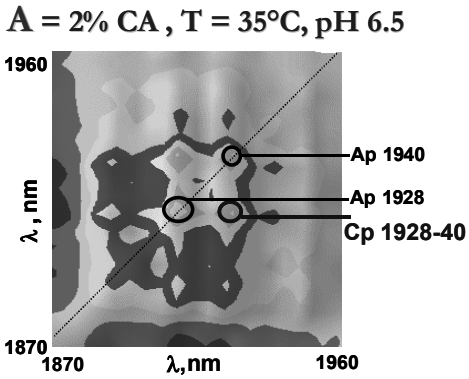

Figure 2. Contour map of the synchronous 2D NIR correlation spectrum of A.

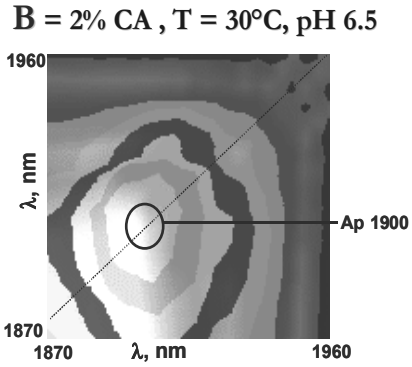

Figure 3. Contour map of the synchronous 2D NIR correlation spectrum of B.

If the pH is lower than A (pH = 6.5), the affinity of the enzyme for the casein micelles will increase leading to an increased reaction velocity. Under these conditions, water molecule rearrangements occur in a different way, and also the water forms (free and bonded) take part in the coagulation process giving contributions different from those detected during the standard process.

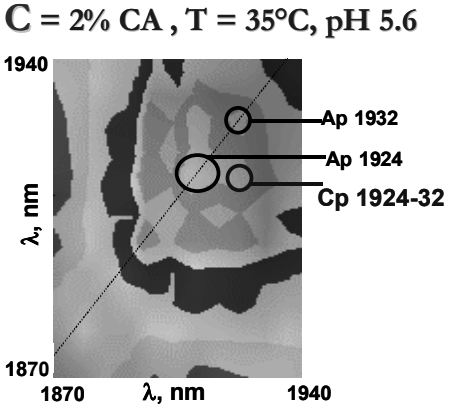

Figure 4. Contour map of the synchronous 2D NIR correlation spectrum of C.

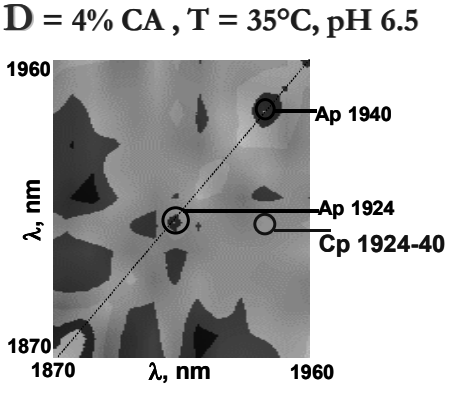

Figure 5. Contour map of the synchronous 2D NIR correlation spectrum of D.

The contour map concerning process D is shown in Figure 5, where the changed process variable is the rennet concentration, which varies from 2% to 4%. This map appears different from A map: two autopeaks at 1924 and 1940 nm are present, although the values of correlation intensity are low. The presence of a cross peak between 1924 and 1940 nm highlights the interactions

interested in the process development. Once more, free and bonded water forms are involved due to the micelle reorganisation after the perturbation of the milk system. On the basis of the low values of correlation intensity assigned to the cross peak between 1924 and 1940 nm, it seems that these rearrangements occur faster than in A, reducing the chances to detect exactly the moment in which the most important changes occur.

The analysis of every modification in the standard process has led to the same interpretation of the results: every change of process variables induces variations in secondary interactions water/water and water/milk constituents.

Conclusions

This work shows that each variation in process variables introduced in the standard system gives 2D maps that are different from the original.

When NIR results are interpreted in terms of water rearrangements and modifications, in addition to the anticipation in monitoring the system destabilisation highlighted by previous works, NIR spectroscopy will provide to be a very powerful tool.

In industrial standard procedures, the detection of these rearrangements can therefore allow the correction for deviations, and the restoration of the desired operational conditions.

References

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