Mid-infrared spectroscopy coupled with 2DCOS and PLS-DA to monitor molecular changes and discriminate camel and cow milk mixtures during coagulation process

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1   Introduction

Camel milk (CaM) is attracting more and more attention from the consumer and the dairy industry due to its high nutritional value [1] and its therapeutic properties [2]. Nonetheless, camel milk has a limited aptitude for technological processing and specifically to coagulation. It has a coagulation time two to three times longer than bovine milk [3]. In this context, different strategies were proposed to manage this issue; one of them is to add milk with high coagulation properties, like cow milk (CM) to CaM milk. In this study, we proposed to study during coagulation the molecular structure effect of adding different proportions of cow milk into camel milk.

2   Material and methods

CaM and CM mixtures were prepared after warming each milk samples to 40 °C in a water bath. The volume fractions (%) of CaM in the different formulations were 100%, 75%, 50%, 25%, and 0%. Milk coagulations were performed at 40 °C (±1°C) by adding 0.25 µL/mL of CHY-MAX® M (Chr. Hansen). MIR spectra were recorded between 3800 and 900 cm^{-1} on the same sample formulation each 5 min during 115 min. The synchronous and asynchronous 2DCOS spectra were calculated by using the generalized 2DCOS algorithm after considering coagulation time as the external perturbation. The 2DCOS analysis was performed separately on two wavelength regions of the five formulations: (1) fat (3000-2800 cm^{-1}) and (2) protein (1700-1500 cm^{-1}). For each MIR wavelength range, the 2DCOS spectra of the five milk formulations were concatenated and analyzed by PLS-DA.

3   Results and discussion

2DCOS coupled to PLS-DA was applied to the MIR spectra in order to extract as much information as possible from the spectral data and attempt to give an order of the molecular structure modifications observed during coagulation. Synchronous 2DCOS maps and their respective auto-peaks resulting from MIR spectra of the protein (figure 1 a,b) and lipid (figure 2 a,b) regions were used to characterize the changes in protein structure and the physical state of triglycerides that occur during milk coagulation. Significant differences (p-value < 0.05) in auto-peak intensities between CM and samples containing at least 50% of CaM in the peak located at 1650 cm^{-1} were observed, which is associated to α-helix vibration [4]. These results highlighted differences in the casein aggregation and therefore casein secondary structure and interactions during milk coagulation [5]. The cross peak symbols of the synchronous (figure 1a, 2a) and asynchronous (figure 1c, 2c) spectra were utilized to identify the sequence of molecular structure changes during coagulation. The results suggested that spectral modifications can be associated to hydrolysis of kappa casein, then the destabilization of

casein micelles and finally their aggregation. Furthermore, the analysis of 2DCOS-MIR maps by PLS-DA allowed for the first time to discriminate between the different formulations with good accuracy. For the synchronous and asynchronous maps in protein range, calibration and prediction models presented no error (0%) and a value of 100% for sensitivity, specificity, and accuracy whatever the milk formulations. Regarding fat range, when considering synchronous spectra values of 100% for sensitivity, specificity, and accuracy, and an error of 0% are obtained for both models. When considering asynchronous spectra, a decrease in the quality of prediction was noted with an increase of the mean error to 13% and a decrease of sensitivity, specificity, and accuracy to 80, 95 and 88%, respectively. These results highlighted the effect of milk formulation on the different synchronous and asynchronous chemical mechanisms that occur during coagulation and that these modifications are sufficiently different to be used to discriminate between the formulations.

4 Conclusion

MIR coupled with 2DCOS was used to follow and describe structure changes, at the molecular level, during coagulation of five milk mixtures. The dissimilarities among the different formulations were revealed on the synchronous 2DCOS and their respective auto-peaks. Moreover, the analysis of the 2DCOS-MIR synchronous and asynchronous maps by PLS-DA yielded good discriminant accuracy between each formulations. 2DCOS-MIR coupled to PLS-DA spectroscopy is a promising method to discriminate milk mixtures and to monitor the structural changes at the molecular level of milk constituents during coagulation process.

5 References

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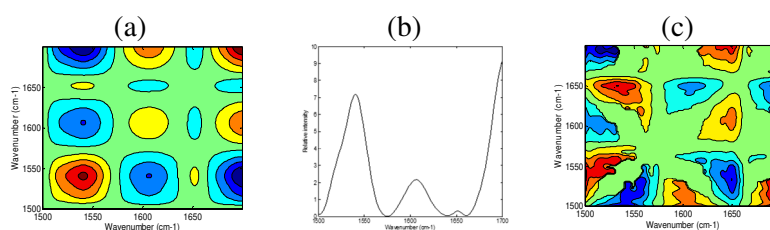
(a)	(b)	(c)

Figure 1- 2DCOS MIR spectra of camel milk (CaM) in protein region: (a) synchronous spectra with their (b) auto-peaks and (c) asynchronous spectra

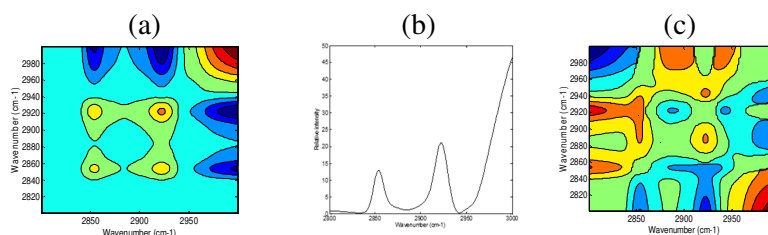| (a)	(b)	(c)

Figure 2- 2DCOS MIR spectra of camel milk (CaM) in fat region: (a) synchronous spectra with their (b) auto-peaks and (c) asynchronous spectra