2D-Cross Correlation Spectroscopy Coupled with Molecular Fluorescence Spectroscopy for Monitoring Coagulation of Camel Milk and Cow Milk Mixtures Coagulation

Oumayma Boukria [1], El Mestafa El Hadrami [1], Françoise Leriche [2] ,Abderrahmane Aït-Kaddour [2]

(1) Université Sidi Mohamed Ben Abdellah, Faculté des Sciences et Techniques, Laboratoire de Chimie Organique Appliquée, Fès, Maroc,

(2) UMR sur le Fromage, Université Clermont Auvergne, VetAgro Sup, INRA, France,

boukriaoumayma@hotmail.com, elmestafa.elhadrami@usmba.ac.ma, francoise.leriche@vetagro-sup.fr,abderrahamane.aitkaddour@vetagro-sup.fr

Introduction

Camel milk is well known for its health benefits and medicinal properties especially in semi-arid and arid areas. It is called desert gold because it is rarely available in the market and sold with high prices. Its adulteration with inexpensive commercial milks has already been started (1).

In the present study, the focus is to describe coagulation of milk mixtures from camel milk (CaM) and cow milk (CM) by a method combing synchronous fluorescence spectra and 2DCOS **(figure 1, 2)** . This was performed in order to appraise the development of an accurate, rapid and feasible analytical method to monitor milk coagulation and to distinguish between different formulations of milk during coagulation because this can be useful for identification of milk adulteration

Materials et Methods

Milk Samples

- Raw **CaM** was collected from a local farm located at Fez, Morocco. Raw **CM** was purchased from a local dairy farm located in Marmilhat, France.
- Five different formulations were prepared by mixing both types of milk. The volume fractions (%) of CaM in the different formulations were 100%, 75%, 50%, 25% and 0%.

Spectroscopic Method and Data Analysis

- **Fluorescence** spectra were recorded between 250 and 550 nm with in a Front Face Synchronous excitation mode (FluoroMax-4) at 40 °C every 5 min during coagulation. For each formulation, three replicates were performed.
- The 2DCOS-synchronous fluorescence spectra were calculated after treatment of the collections of coagulation time-dependent dynamic spectra with 2DCOS analysis method by using a home software program in MATLAB.

Results and discussion

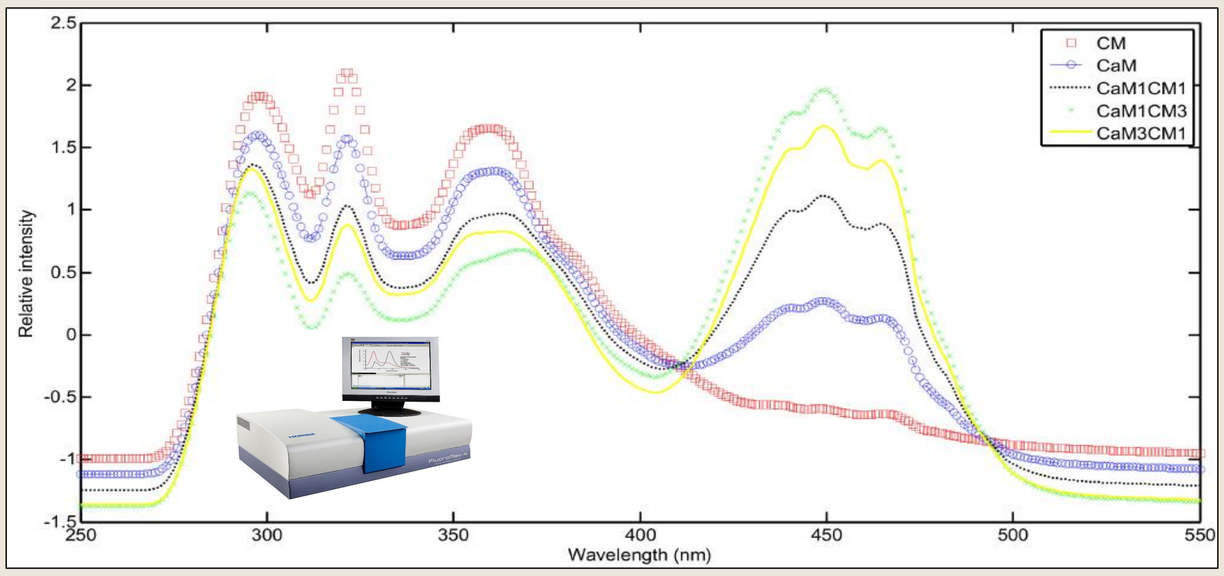

Fig.1. Normalized mean synchronous fluorescence spectra recorded during coagulation of CM, CaM and their mixtures.

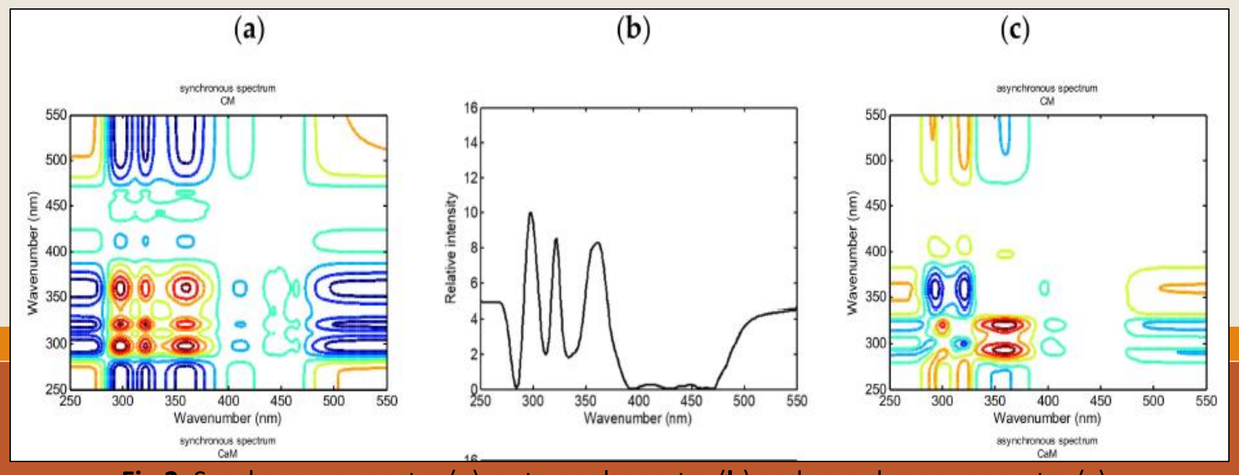

Fig.2. Synchronous spectra (**a**), auto-peak spectra (**b**) and asynchronous spectra (**c**).

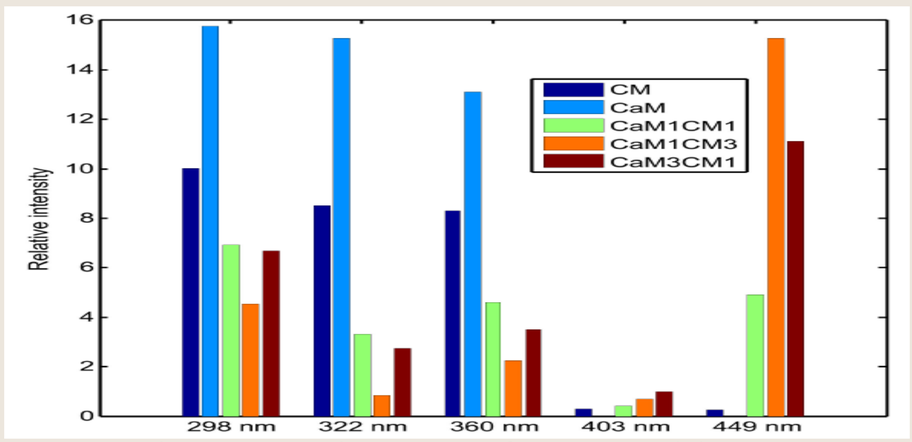

Fig.3. Relative auto-peak intensities of CM, CaM and their mixtures.

- Four bands can be observed centered **(Fig. 1)** on 297 nm (emission 377 nm), 321 nm (emission 401 nm), 360 nm (emission 440) and 449 nm (emission 529 nm). Those bands were previously identified, in milk and cheese, and were assigned to tryptophan, vitamin A and riboflavin compounds, respectively.
- A positive cross-peak (in red or green color area in **Fig. 2**) indicates a simultaneous increase or decrease of different fluorescence bands under the coagulation time. In contrast, a negative cross-peak (blue area color in **Fig. 2**) represents the coordinated changes of band intensities in the opposite directions.
- The asynchronous spectra further revealed the sequence of the molecular fluorescence modification during milk coagulation (structure, interaction and environment). Below the diagonal line of the asynchronous maps of the different milk formulations, five peaks were identified at 298, 322, 360, 403 and 450 nm. The different cross-peaks formed by those bands are presented in **Table 1**.

	Sign of Identified Peak				
Peak Position (nm)	298 nm	321 nm	360 nm	403 nm	450 nm
298 nm	+	+ (-)	+ (+)	- (-)	+ (+)
322 nm		+	+ (+)	- (-)	+ (+)
360 nm			+	-	+ (-)
403 nm				+	+ (+)
450 nm					+

Table 1. The results of two-dimensional correlation spectroscopy (2DCOS)

The dissimilarities among the different formulations are precisely noticed on the synchronous 2DCOS fluorescence spectra **(Fig. 2a)**. In addition, according to the cross-peak symbols in synchronous and asynchronous spectra **(Table 1)**, the speed of modification in the fluorescence molecules (riboflavin, protein and vitamin A) during coagulation time corroborated with common coagulation phenomena usually reported during chymosin coagulation.

Conclusion

Molecular fluorescence spectroscopy associated with 2DCOS spectroscopy was used to monitor modifications occurring during the coagulation of different milk formulations based on CM and CaM mixtures. The method demonstrated that this strategy successfully discriminates milk mixtures and monitors molecular structure modifications occurring during coagulation process. Efficient, effective and non-destructive, this emerging tool based on fluorescence and 2DCOS spectroscopy, could also be proposed in the near future to identify adulteration of CaM by CM before and during processing.

References:

1- Mabood et al. 2017. FT-NIRS coupled with chemometric methods as a rapid alternative tool for the detection & quantification of cow milk adulteration in camel milk samples. Vibrational Spectroscopy 92 (2017) 245-250.