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Delineation of molecular structure modification during coagulation of mixed camel and cow milk by mid-infrared spectroscopy and parallel factor analysis

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Abstract

Molecular structure modifications of camel milk (CaM) and cow milk (CM) mixtures during coagulation were investigated combining mid-infrared (MIR) spectroscopic monitoring with Parallel Factor Analysis (PARAFAC) and particle-size measurements. To evaluate the structure evolution at the molecular level, five different milk formulations were prepared using the following volume fractions of CaM in the mixtures: 100, 75, 50, 25, and 0. Regarding MIR spectroscopy, wavelength ranges located between 3,000 and 2,800 cm^{-1} corresponding to fatty acids; from 1,700 to 1,500 cm^{-1}, related to amide I and II bands; and in the 1,500–900 cm^{-1} range called the fingerprint region were considered for the characterization of milk coagulation kinetics. MIR spectroscopy with PARAFAC was allowed for the identification of modifications at the molecular level depending on the coagulation time and milk composition. This was also confirmed by canonical correlation analysis, which demonstrated a high degree of correlation between the casein particle-size distributions and MIR spectra measured during coagulation.

Novelty impact statement: PARAFAC coupled with MIR spectroscopy were successfully used to monitor coagulation of CM, CaM, and their mixtures (CaM, CM, 1CaM:1CM, 1CaM:3CM, 3CaM:CM1; v/v). PARAFAC coupled with MIR spectroscopy were successfully used to differentiate between physicochemical phenomenon occurring during coagulation of different mixtures of CM, CaM and their mixtures. Canonical correlation analysis (CCA) of MIR spectra and particle-size distribution during milk coagulation demonstrated a strong relationship between the formation of casein aggregates and the variation of the MIR spectral data.

1 | INTRODUCTION

Mixing milks from different species can be strategized to increase consumption of nonbovine milks and enable consumers and dairy companies to benefit from the nutritional and technological advantages of these mixes (Boukria, El Hadrami, Boudalia, et al., 2020; Faye & Konuspayeva, 2012; Park, 2017). It is particularly noted that camel milk (CaM) is of high nutritional quality, for example, it contains three times more Vitamin C, a greater variety of minerals (e.g., K^{+}, Cu^{2+}, and Mn^{2+}), and more essential and polyunsaturated fatty acids than cow milk (CM) (Farah et al., 1992; Sawaya et al., 1984). CaM has also been shown to exhibit properties that manage chronic ailments (Khalesi et al., 2017). Regarding this strategy, it is important to characterize the quality features of products made by mixing

milks from different animal species in order to develop products with the proper characteristics that will attain satisfactory consumer acceptance.

Cheese texture is considered one of the most significant attributes of cheese identification and quality (Creamer & Olson, 1982; Euston et al., 2002; Jack et al., 1993; Loudiyi & Aït-Kaddour, 2018), and milk coagulation is the primary step in the development of texture in most dairy products (e.g., cheeses and yoghurts). Therefore, good management of this operation is essential for obtaining a cheese that features the targeted texture. Among other factors, it was reported that textural characteristics of cheese are affected by their structural properties, composition, and fat distribution, all of which are highly dependent upon the manufacturing process (Lobato-Calleros et al., 1998). The management of the coagulation step is also important because the microstructure of dairy products is closely related to other quality features of cheeses, such as physicochemical properties, flavor, color, nutritional profile, and nutrient bioavailability (Lamichhane et al., 2018).

Nowadays, different techniques are available to investigate the molecular structure and microstructure of dairy products in detail. Within the last decade, different applications of mid-infrared (MIR) spectroscopic monitoring for food analysis have been proposed in this context. In fact, for milk and dairy products, MIR spectroscopy has been used to monitor texture changes during cheese ripening (Kulmyrzaev et al., 2005; Loudiyi et al., 2017), to investigate the effect of NaCl substitution by KCl on the molecular structure of cheese (Loudiyi & Aït-Kaddour, 2018), and to examine the effect of heat treatment on rheological properties of cheese (Boubellouta & Dufour, 2012).

Discerning the effect of mixing milk from different species on molecular structure and the location and interaction of components (e.g., fat, proteins) during manufacture is essential for predicting quality attributes of dairy products, particularly cheese. However, it is generally understood that MIR spectroscopy has never been utilized to study the effect of mixing different milks on the molecular structure during coagulation. Therefore, the aim of the present work is to evaluate changes at the molecular level during rennet coagulation of different CaM and CM mixtures via MIR spectroscopy combined with chemometric tools and particle-size features.

2 | MATERIALS AND METHODS

2.1 | Milk and milk coagulation

Holstein raw CM (500 ml) was purchased in 2019 from a dairy farm of 30 animals located in Marmilhat, France, and raw CaM (500 ml) was acquired in the same year from a local farm of eight Sahraoui breed camels located in Fez, Morocco. The average composition of the milk samples was analyzed via the Gerber method for fat, the Kjeldahl method for protein, and the colorimetric method for lactose according to their normative references (ISO 488:2008, ISO 8968-1:2014, and ISO 26462:2010). The average composition of CM was 3.10% fat, 3.21% protein, and 4.16% lactose. For CaM, the average composition was 4.77% fat, 2.96% protein, and 4.82% lactose. The CaM and CM mixtures were prepared after each milk sample was warmed to 40°C using a water bath and gently mixed by hand for at least 1 min. The volume fractions (%) of CaM in the different formulations were performed according to previous reviews of Boukria, El Hadrami, Boudalia, et al. (2020) and Boukria, El Hadrami, Sameen, et al. (2020), which were fixed at 100% (i.e., CaM), 75% (i.e., 3CaM:1CM, v/v), 50% (i.e., 1CaM:1CM, v/v), 25% (i.e., 1CaM:3CM, v/v), and 0% (i.e., CM). Milk coagulations were performed at 40°C (±1°C) using 0.25 μl/ml of CHY-MAX® M (Chr. Hansen, Denmark) containing 50 mg/L of chymosin. After the addition of chymosin, the milk samples were gently mixed by hand for approximately 10 s and then deposited in a pre-warmed (40°C) measurement cell that had been previously installed in the spectral MIR device. The temperature was adjusted via a Specac Series 4000 temperature controller (Eurolabo, Paris, France).

2.2 | Size distribution of casein micelles

Particle-size distributions of CaM, CM, and their mixtures during coagulation were measured by a laser diffraction instrument (Malvern Mastersizer S, Malvern Instruments, Worcestershire, England, United Kingdom) at 23°C (±1°C). To overcome multiple scattering effects during measurement, the samples were diluted with a solution of imidazole-acetate buffer (5 mM, pH7), and in order to ensure homogeneity, the solutions were continuously stirred during measurement. Particle-size features were measured using five factors (D [3,2], D [4,3], Dx [10], Dx [50], and Dx [90]) (Amador-Espejo et al., 2014). The D [3,2] factor represents the surface-weighted mean, and the D [4,3] factor represents the volume-weighted mean, with the first parameter being more sensitive to the distribution of fines and the second to the coarse end of the distribution (Fleming et al., 2017). The Dx [10], Dx [50], and Dx [90] correspond to the percentiles of volume distribution, which are useful in determining how small, average, or large the size distribution of particles is. D10 is the value wherein 10% of the distribution is smaller than that value in microns. The D50 is the median where 50% of the distribution is smaller than that size and 50% larger. The D90 is the value where 90% of the distribution is smaller than that size in microns. Particle-size properties were calculated as the average of duplicate measurements (Amador-Espejo et al., 2014).

2.3 | Spectral acquisition

During milk coagulation, MIR spectra were recorded using a Tensor II Series Fourier Transform Spectrometer (Bruker, Billerica, MA, USA) containing a thermostated ATR ZnSe accessory (six reflections, incident angle 45°). MIR spectra were recorded between 3,800 and 900 cm^{-1} at a resolution of 4 cm^{-1} with OPUS software Version 7.5 (Bruker). Thirty-two scans were recorded per spectrum in order to

improve the signal-to-noise ratio. All spectra were recorded on the same sample formulation every 5 min, for 115 min, at a temperature of 40°C. The temperature was regulated using a Specac Series 4000 temperature controller (Eurolabo). Three replicates were recorded per milk formulation (CaM, 3CaM:1CM, 1CaM:1CM, 1CaM:3CM, and CM).

2.4 | Statistical analysis

Parallel factor analysis (PARAFAC) is a generalization of principal component analysis (PCA), which decomposes multiway data. This chemometric tool decomposes an N-order array into the sum of the outer products of the N loading components being observed. An important advantage of using PARAFAC instead of unfolding PCA is that the estimated models are very simple in a mathematical sense and are, therefore, easier to interpret (Bro, 1997). The number of PARAFAC components necessary to reconstruct the data is an important parameter that can be determined by several methods. In the present study, the core consistency diagnostic was chosen. A drop in the core consistency from a high value (above approximately 60%) to a low value (below approximately 50%) indicates that an appropriate number of components has been attained.

Super Vector Machine (SVM) is a well spread supervised method that has demonstrated a good ability to be applied in both linear and nonlinear data. The aim of SVM is to find an optimal hyperplane that correctly separates objects belonging to different classes by preserving the greatest possible portion of points that belong to the same class on the same side of the hyperplane while maximizing the distance of either class from the hyperplane. Radial basis function was chosen as the kernel function of SVM, and the SVM parameters were optimized by a grid search procedure and venetian blind cross validation (number of data splits = 10, samples per blind = 1).

The canonical correlation analysis (CCA) method, a predictive procedure in chemometrics, was applied to the preliminary results above in order to understand the relationship between the two data sets (spectral data and size of particle aggregates). This analysis was conducted by calculating the canonical correlation factor between the data sets (spectra and particle-size distribution).

PARAFAC, SVM, and ACC analysis and data preprocessing were performed in MATLAB R2012b (The MathWork, Natick, Massachusetts, USA) coupled with the PLS-toolbox 852 (Eigenvector Research, Manson, Washington, USA).

3 | RESULTS AND DISCUSSION

3.1 | Description of the MIR spectra

The MIR spectra of the five milk formulations (CaM, CM, 1CaM:1CM, 1CaM:3CM, 3CaM:CM1; v/v) involved in the study after smoothing by savgol method (Savitzky & Golay, 1964) (second-order polynomial applied within a sliding of 15-points spectral window) and normalization by the Standard Normal Variate method (Barnes et al., 1989) are presented in Figure 1.

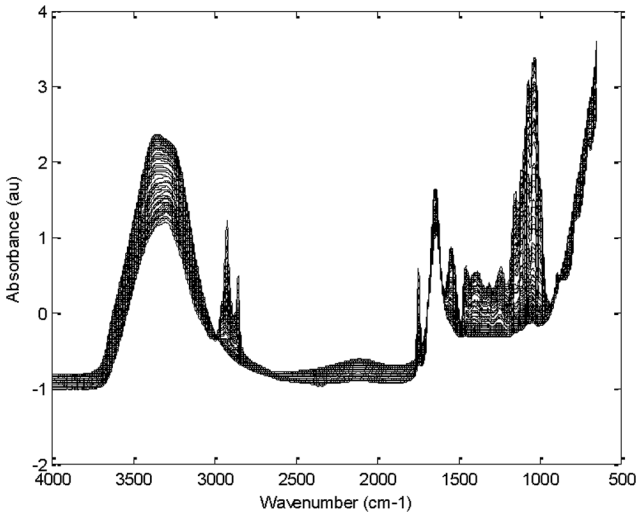

FIGURE 1 MIR spectra recorded during enzymatic coagulation of the different mixtures of camel milk and cow milk

The spectra exhibited different absorbance bands and peaks located in the 3,800–3,000, 3,000–2,800, 1,700–1,500, and 1,500–900 cm^{-1} regions. The 3,800–3,000 cm^{-1} region is a band designated to vibration of water (the O-H stretching mode) (Chen et al., 1998). The two bands at 2,923 and 2,853 cm^{-1} in the 3,000–2,800 cm^{-1} region are assigned to methylene anti-symmetric and symmetric stretching (Casal & Mantsch, 1984), respectively. Absorptions in the 1,700–1,500 cm^{-1} region are characteristic of peptide and protein bands. The absorption bands around 1,641 cm^{-1} and 1,547 cm^{-1} are generally attributed to the amides I and II, respectively (Bellamy, 1975; Mazerolles et al., 2001). The 1,500–900 cm^{-1} range is referenced as the fingerprint region and exhibits different absorption bands (O-C-H, C-C-H, C-O-H, P=O and fatty acids) (Bellamy, 1975; Meurens et al., 2005; Paradkar et al., 2002; Sivakesava & Irudayaraj, 2001).

3.2 | Parallel factor analysis

Conclusions about the effect of both milk formulations and coagulation time on spectral changes are not evident by direct observation of the spectra despite the assignment of bands. In order to precisely identify chemical and physicochemical information from spectra and provide in-depth analysis, the mean of triplicate data for each formulation was arranged in three-way matrix in which the x, y and z dimensions was assigned wavenumbers for MIR data (2,347), time mode (24), and sample formulation (5), respectively.

The implementation of PARAFAC gave three outcomes (i.e., Modes 1, 2, and 3), each one related to the loading of a different mode. Mode 1 gave the kinetic mode loadings related to scores indicating how the spectra varied during coagulation time (kinetic of coagulation). Mode 2 corresponded to the fingerprint mode (wavelength or wavenumber variables) and indicated which variable was

responsible for the variation in modes 1 and 3. Mode 3 showed the loading profiles related to the formulation factor (i.e., the ratio of CaM to CM).

It is generally held that the most important information provided by MIR spectra is located in three wavelength ranges (i.e., 3,000–2,800, 1,700–1,500, and 1,500–900 cm^{-1}). PARAFAC was performed separately to the amide I/amide II (1,700–1,500 cm^{-1}), C-H stretching (3,000–2,800 cm^{-1}), and fingerprint (1,500–900 cm^{-1}) wavelength ranges (Boubellouta et al., 2011) in order to streamline the interpretation of the spectral loading modes, which is a complex process when the entire spectral wavelength range is considered.

The PARAFAC results applied to the amide I/amide II range are presented in Figure 2a–c. The similarity map reveals a discrimination between scores on the first component (Figure 2a) according to coagulation time.

Regarding the spectral pattern of component one (Figure 2b), positive peaks are seen at 1,628, 1,566, 1,549, 1,530, and 1,516 cm^{-1}, and a negative band, which was observed between 1,700 and 1,643 cm^{-1}, presented two minima at 1,668 and 1,556 cm^{-1}. This pattern indicated a blue shift of the amide I band and an increase in the amide II width during coagulation time. The changes in the spectra recorded during milk coagulation can be interpreted as changes in casein-bound water. The spectral pattern corresponding to component 2 (Figure 2b) displays characteristic bands at 1,650 and 1,540 cm^{-1} roughly corresponding to the mean spectrum of proteins, which suggests that the observed changes in the spectra were

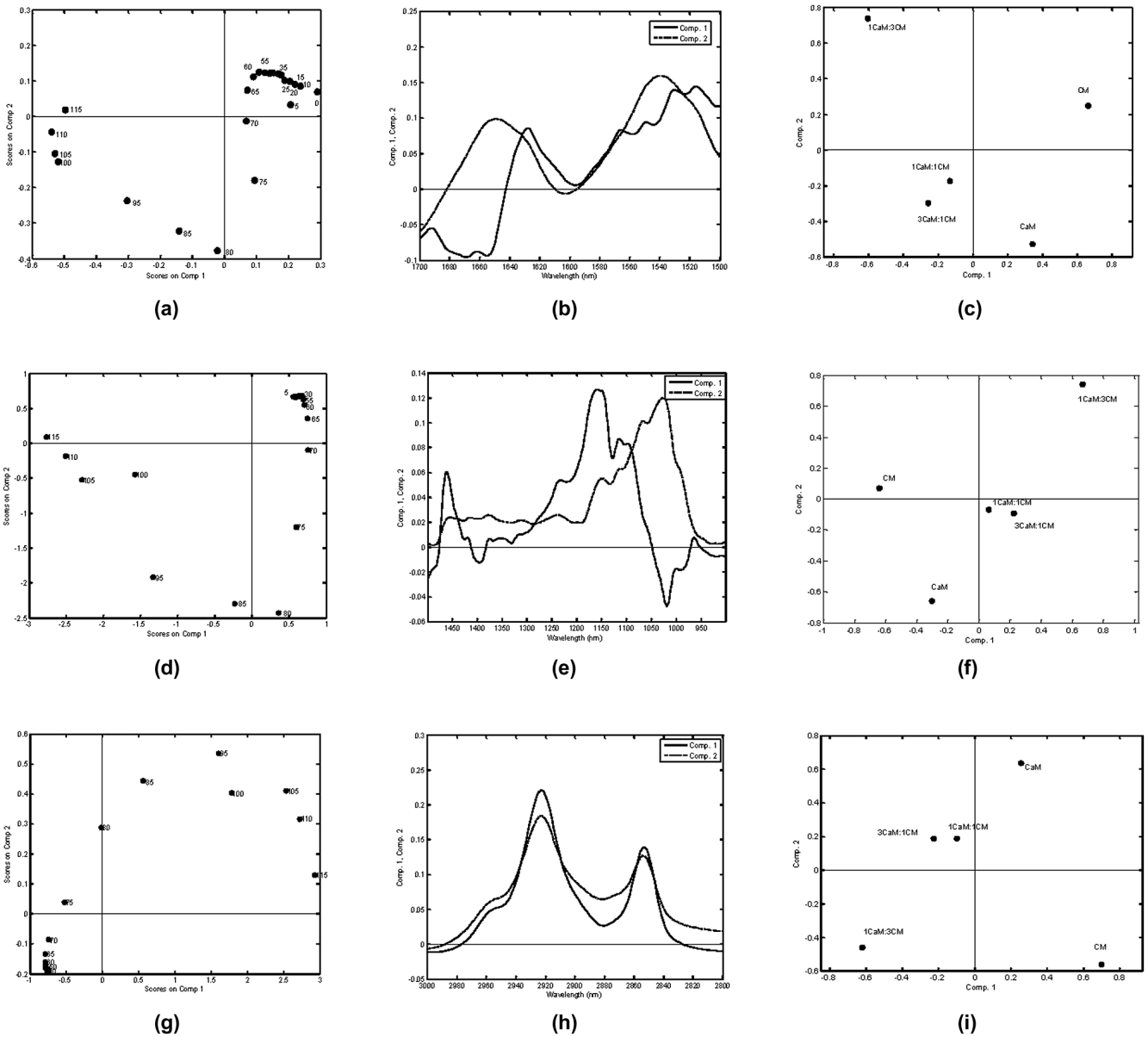

FIGURE 2 Two-component PARAFAC model derived from the MIR data (a–c: amide I/amide II; d–f: fingerprint and g–i: fat range) recorded during milk coagulation of the different milk formulations containing a mixture of camel milk (CaM) and cow milk (CM). (a, d, and g) Kinetic mode, fingerprint mode (b, e, and h) and formulation mode (c, f, and i). Milk formulations are noted as follows: CaM, 3CaM:1CM, 1CaM:1CM, 1CaM:3CM, and CM (v/v)

only related to a difference in absorbance over this particular spectral range. This finding indicates that the most important information is provided by the first component.

The information related to the mode of the samples (Figure 2c) shows that samples formulated with pure CaM and pure CM displayed positive values for component 1, while samples containing a mixture of CaM and CM milks (i.e., 1CaM:1CM, 1CaM:3CM, and 3CaM:1CM) returned negative values. In regards to component 2, samples containing more than 75% CM (1CaM:3CM and CM) produced positive scores while the other samples (i.e., CaM, 3CaM:1CM, and 1CaM:1CM) gave negative scores. These observations demonstrate that mixing CaM and CM affects the protein network structure developed during milk coagulation. These results have been corroborated by Shahein et al. (2014). PARAFAC results of the analysis of the fingerprint region are presented in Figure 2d–f. Figure 2d contains a similarity map of components 1 and 2 that depicts an inverted bell-shaped curve, which was previously reported as representing milk coagulation by acidification procedure (Boubellouta et al., 2011).

Concerning component 1, positive scores were noted between 0–80 min, while negative ones were observed from 85 to 115 min. The spectral patterns for the first component (Figure 2e) exhibit different positive peaks at 1,460, 1,419, 1,233, 1,159, 1,115, 1,095, and 963 cm^{-1} and negative ones at 1,396, 1,019, and 988 cm^{-1}. With regards to component 2, only positive peaks were noted at 1,147, 1,112, 1,066, and 1,025 cm^{-1}. The information related to the mode of the samples (Figure 2f) shows that samples formulated with pure CaM and CM resulted in negative values for component 1, while samples containing a mixture of CaM and CM (i.e., 1CaM:1CM, 1CaM:3CM, and 3CaM:1CM) produced positive values.

For component 2, it was observed that samples containing more than 75% CM (1CaM:3CM and CM) returned positive scores while the other samples (i.e., CaM, 3CaM:1CM, and 1CaM:1CM) delivered negative scores. These results imply that mixing CaM and CM impacts coagulation time. Therefore, mixing CaM with CM can modify the coagulation kinetic of the initial milk. This corroborates the previous observation that the production of cheese from CaM is difficult under natural conditions due to the fact that CaM does not measurably coagulate because of poor coagulation properties (Farah & Ruegg, 1989) related to the primary structure of κ-casein and the size of the casein micelles.

With regards to the region of the lipids band, Figure 2g shows a similarity map of components 1 and 2. The scores on the map form a bell-shaped curve for the fingerprint region. Regarding components 1 and 2, the spectral patterns (Figure 2h) exhibit two prominent positive peaks at 2,930 and 2,850 cm^{-1} and a shoulder at 2,960 cm^{-1}. The information related to the samples mode (Figure 2i) shows that the first component discriminated the pure milks (i.e., CaM and CM) from the mixed CaM and CM formulations. Concerning the second component, it can be observed that the formulations were almost separated depending on the concentration of the CM content. Samples containing more than 75% CM (1CaM:3CM and CM) delivered negative scores while the other samples (i.e., CaM, 3CaM:1CM, and 1CaM:1CM) presented positive scores. Indeed, the value of component 2 decreased with an increase of CM content in the formulation. Loading profiles 1 and 2 are similar to the mean spectra observed in this wavelength range suggesting that the observed changes in the spectra are related to absorbance difference over the whole spectrum. The modification of the intensity of the lipids band is generally assigned to a modification in the physical state of triglycerides (Boubellouta et al., 2011; Loudiyi et al., 2017). These findings underline an alteration of lipid-protein interactions during coagulation.

3.3 | Casein micelle aggregation and correlation with spectral data

The five size features (D [4,3], D [3,2], Dx(10), Dx(50), and Dx(90)) measured during coagulation of the five milk formulations (CM, CaM, 1CM:1CaM, 1CM:3CaM, and 3CM:1CaM) were concatenated in one matrix and analyzed via SVM. This was performed in order to identify the existing difference between formulations during coagulation time. The SVM results, confusion table, accuracy, sensitivity, and specificity factors for each kind of milk formulations are summarized in Table 1. Confusion matrix analysis for the classification of the milk formulations resulted in four states: true positive (TP, milks correctly assigned to the intended class), true negative (TN, milks correctly assigned to the intended class), false positive (FP, milks incorrectly assigned to the intended class) and false negative (FN, milks incorrectly assigned to the intended class). The statistical features sensitivity (proportion of positive cases that were correctly identified: TP/(TP + FN)), specificity (proportion of negatives cases that were classified correctly: TN/(TN + FP)), and accuracy (proportion of samples which were correctly classified: (TP + TN)/(TP + TN + FP + FN)) were used to analyze system performance. The statistics of the SVM model (Table 1) presented high values (i.e., 68.4 < statistics < 96.5%) for specificity, sensitivity, and accuracy. These results highlighted that a clear difference in the casein aggregates between samples can be observed during coagulation.

To identify the relationship between infrared spectra and particle-size distribution and to confirm the potential of MIR spectroscopy to reveal molecular changes occurring during coagulation, CCA was applied jointly to the scores obtained after PCA of MIR spectra and particle size distribution of aggregates. The number of PCs before performing CCA was defined when PCA explained 99% of the variance. Three, five, and five PCs were determined for the MIR fat region (3,000–2,800 cm^{-1}), the MIR amide I and II region (1,700–1,500 cm^{-1}) and the fingerprint region (1,500–900 cm^{-1}) respectively. The amide I/II and fingerprint regions showed the highest square correlation factors (Table 2). This result is consistent with the process of milk coagulation kinetic observed after adding chymosin: κ-casein hydrolysis, casein micelle disruption, and aggregation (Dalgleish, 2011). These correlations indicate that the canonical variates provided description visualization of the samples both from the spectral data and the size aggregates of the casein micelles.

TABLE 1 The performance summary of the SVM model after cross-validation

	Confusion table					Statistics (%)		
	1CaM:1CM	1CaM:3CM	3CaM:1CM	CM	CaM	SP	SE	AC
Predicted as 1CaM:1CM	98	4	10	5	6	95.2	75.4	90.2
Predicted as 1CaM:3CM	13	111	2	30	0	91.3	85.4	91.2
Predicted as 3CaM:1CM	10	0	101	4	9	95.6	77.7	92.0
Predicted as CM	7	14	4	89	2	94.8	68.4	90.0
Predicted as CaM	2	1	13	2	113	96.5	86.9	94.6
Predicted as unassigned	0	0	0	0	0			

Note: Milk formulations are noted as follow: CaM, 3CaM:1CM, 1CaM:1CM, 1CaM:3CM, and CM (v/v).
Abbreviations: AC, accuracy; CaM, camel milk; CM, cow milk; SE, sensitivity; SP, specificity; SVM, Super Vector Machine.

TABLE 2 Canonical correlation factor obtained after CCA calculated between spectral and particle-size features

Technique	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8
MIR								
Fat range (3,000–2,800 cm^{-1})	0.47	0.34	0.19	–	–	–	–	–
Protein range (1,700–1,500 cm^{-1})	0.72	0.52	0.39	0.26	0.12	–	–	–
Finger print range (1,500–900 cm^{-1})	0.61	0.52	0.25	0.24	0.14	–	–	–

Abbreviation: CC, canonical component; CCA, Canonical correlation analysis.

4 | CONCLUSIONS

MIR spectroscopy was used to investigate modifications that affect molecular structure during milk mixture coagulation. The results provided distinct information related to milk components during coagulation, which, correspondingly, provided the means to gather further data associated with changes in the molecular structure of both the milk components being studied as well as interactions within the various milk formulations being observed. MIR spectroscopy permitted the identification of a variation of the coagulation kinetic that was dependent upon the initial milk formulation. Therefore, it is concluded that spectroscopic methods, like MIR spectroscopy, combined with chemometric tools, such as PARAFAC have the potential to characterize structural changes at the molecular level during milk coagulation. This finding was reinforced by CCA that demonstrated a strong relationship between the formation of casein aggregates during coagulation and the spectral data.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Oumayma Boukria: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Software; Validation; Visualization; Writing-original draft. **Jian Wang:** Methodology; Validation; Visualization. **Jasur Safarov:** Methodology; Resources; Validation; Visualization. **Adem Gharsallaoui:** Methodology; Resources; Validation. **Françoise Leriche:** Conceptualization; Supervision; Validation; Visualization. **El Mestafa El Hadrami:** Conceptualization; Validation; Visualization. **Abderrahmane Ait Kaddour:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Software; Supervision; Validation; Visualization; Writing-original draft.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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