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ORIGINAL
RESEARCH

Investigation of heat-acid induced coagulation behaviour of whole milk systems employing front-face fluorescence spectroscopy

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The heat-acid-induced coagulation behaviour of whole milk system (buffalo, cow and mixed milk) was studied by steady-state fluorescence spectroscopy exploiting tryptophan as a marker molecule. The varied molecular environment of tryptophan residues in the raw milk systems exerted different fluorescence properties. During the coagulation process, cow milk exhibited significant quenching and bathochromic shift in the emission spectra which was attributed to the low buffering ability and high hydration capacity of cow milk caseins. The results indicated that tryptophan residues of cow milk experienced more dynamic environment throughout the reaction as compared to buffalo milk.

Keywords Coagulation, Raw milk, Tryptophan, Fluorescence spectroscopy.

INTRODUCTION

Heat denaturation of proteins involves their unfolding with the loss of biological activity due to breakdown of 2D hydrogen bond networks present in the water surrounding the protein structures (Cheng and Cui 2018). During denaturation , protein structure originally loosens and unfolds into residual conformations, enabling these structures to absorb more water thereby increasing the hydration capacity with respect to the exposed water surface by up to 50% (Wiggins 1997). Understanding the denaturation, aggregation, heat stability and state of water hydration of the milk proteins during dairy product manufacturing is therefore of both theoretical and commercial importance to food scientists.

Milk is a complex system comprising of various fluorescent molecules, such as riboflavin, aromatic amino acids and nucleic acids (Herbert *et al*. 2000). The three aromatic amino acids, tryptophan (Trp), tyrosine and phenylalanine, are the cause of intrinsic fluorescence of milk proteins. Of these, Trp dominates the fluorescence owing to its high coefficient of extinction (innate dominant fluorophore). Trp residue's fluorescence offers data on protein structure (Fox 1989). Fluorescence is dependent on how Trp is subjected to proteins and solvent polarity in the three-dimensional association (Lakowicz *et al*. 1983). Changes in Trp emission spectrum occur often due to conformational changes, connection of subunits, substrate binding or denaturation of milk proteins (Longworth 1983; Engelborghs 2001). Trp emissions are extremely susceptible to its local surroundings and thus are often used as a marker for understanding the alterations in protein structure and protein associations in cheese composition. Trp emission spectra can be directly linked with protein content, acid concentration and extent of heat treatment of milk. Several researchers have studied structural variations in the proteins in soft-cheese samples through fluorescence spectroscopy in order to explain the chemical composition, process conditions, geographical origin and effects of season (Herbert *et al*. 2000; Dufour *et al*. 2001; Karoui and Dufour 2003; Kulmyrzaev *et al*. 2005; Karoui and Dufour 2006; Karoui *et al*. 2007).

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Trp content is lower in buffalo milk (0.053 g/100 g) as compared to cow milk (0.075 g/100 g) (USDA 2006). Buffalo milk contains higher proportions of casein and whey proteins than cow milk. The buffalo casein micelles are bigger (110–160 nm) than those in bovine milk (70–110 nm) (Khedkar *et al*. 2015). Alpha s_1 casein (αs_1) and kappa casein (k-CN) contents are higher in buffalo milk whereas, alpha s_2 casein (αs_2) and beta casein (β-CN) are higher in cow milk (Misra *et al*. 2008). Higher αs_1-CN and lower β-CN are associated with increased coagulation time and gel firming duration by trapping calcium ions due to which the buffalo milk gel is firm and has strong coagulation properties. Higher content of β-CN in cow milk decreases the coagulation time as well as the firmness of the gel which gives the gel a weak coagulation property (Dalgleish 2011). Buffalo milk has higher levels of calcium, magnesium and inorganic phosphate compared to cow milk. Both milk varieties contain more or less similar levels of soluble calcium and inorganic phosphate but total calcium and inorganic phosphate in buffalo milk are substantially higher. This indicates that the calcium and inorganic phosphate associated with casein micelles are higher in buffalo milk than in cow milk and provides higher heat stability (Ahmad *et al*. 2008).

In Indian milk industry, cow milk and buffalo milk are often mixed together for product manufacturing like *Chhana* (Chandan 2007). *Chhana* commonly known as Indian cottage cheese is an important dairy product of Indian subcontinent. *Chhana* is manufactured by a heat-acid-induced coagulation process. *Chhana* production involves casein coagulation with entrapped fat and water as well as water soluble components by the addition of a suitable coagulant (citric acid, lactic acid, calcium lactate, etc.) to hot milk. However, there are no detailed scientific studies on investigating the changes occurring at molecular level during the process of *chhana* making from whole milk (cow, buffalo and mixed milk). In the present study, fluorescence spectroscopy has been used to study the qualitative changes in milk protein structures (casein and whey proteins) by monitoring thermal and acidic denaturation of the whole milk proteins at molecular level and discriminate between buffalo, cow and mixed milk samples under different conditions (raw milk, heated milk and heated milk with coagulant). To understand the effect of process parameters on the whole milk protein system, Trp has been used as a marker molecule to plot the variation in buffalo milk (BM) as compared to cow milk (CM) and mixed milk (MM) systems at three different conditions: (i) ambient condition (30 °C); (ii) heating condition (heating up to 90 °C and cooling down to 70 °C) and (iii) acidification condition (addition of 1% citric acid as coagulant at 70 °C). To our knowledge, this would be the first study of heat-acid-induced coagulation behaviour of whole milk systems through fluorescence spectroscopy.

MATERIALS AND METHODS

Sample collection

Fresh raw BM (Murrah breed, 14–15 L/day production) and fresh raw CM (Jersey breed, 10–12 L/day production) were procured from local dairy farm from Dhanas, Chandigarh, India in the winter season (January-February, 2019). Mixed milk (MM) samples were prepared by mixing both the milk samples in desired proportions (75:25, 50:50, 25:75 by weight) (Table 1) for further analysis (moisture, solid-not-fat (SNF), total solids, lactose, fat and protein contents). Ambient and milk temperatures were recorded at every stage. The raw milk parameters were analysed and recorded before processing.

Analysis of raw milk

Determination of moisture, total solids, fat, and protein contents

Moisture, total solids, fat and protein contents were measured according to BIS (1981). All measurements were carried out in triplicate, and chemicals of analytical grade were used.

Chhana manufacturing process

Chhana was prepared from the BM, CM and mixed milk (MM_1, MM_2 and MM_3) following the method described by Kundu and De (1972) with certain modifications. The modifications included different coagulating temperature, pressing time and storage conditions. For each experimental set of preparing *chhana* samples, 500 g milk was heated for 7–10 min till the temperature reached 90 ± 1 °C followed by cooling to coagulation temperature for 5–7 min till the temperature dropped at 70 ± 1 °C. 70 g citric acid solution (1% w/v concentration) was then incorporated slowly with constant stirring. Coagulation took place immediately leading to separation of coagulated mass from the whey. Fluorescent spectra acquisition of the milk (in-situ) samples was carried out by mimicking the *chhana* manufacturing process parameters.

Fluorescence spectroscopy of raw and heated milk

One mL of the milk sample was used to record emission spectra at each stage of milk coagulation procedure (raw

Table 1 Details of milk samples.[a]

	Proportion (% weight)				
Milk type	*BM*	*MM_1*	*MM_2*	*MM_3*	*CM*
BM	100	75	50	25	0
CM	0	25	50	75	100

[a]BM, buffalo milk; CM, cow milk; MM, mixed milk (MM_1, mixed milk 1; MM_2, mixed milk 2; MM_3, mixed milk 3).

Table 2 Composition of BM, CM and MM (MM_1, MM_2, MM_3).[a]

Constituent (%)	*BM*	*MM_1*	*MM_2*	*MM_3*	*CM*
Moisture content	82.91 ± 0.18^{d}	83.99 ± 0.20^{c}	84.84 ± 0.14^{b}	85.23 ± 0.15^{a}	85.57 ± 0.17^{a}
Total solids	16.87 ± 0.09^{a}	15.85 ± 0.05^{b}	15.01 ± 0.03^{c}	14.67 ± 0.17^{d}	13.96 ± 0.13^{e}
Fat	6.73 ± 0.06^{a}	6.19 ± 0.13^{b}	5.94 ± 0.09^{c}	5.41 ± 0.12^{d}	4.92 ± 0.06^{e}
SNF	9.88 ± 0.13^{a}	9.44 ± 0.07^{b}	8.89 ± 0.14^{c}	8.38 ± 0.22^{d}	8.09 ± 0.08^{e}
Protein	4.02 ± 0.03^{a}	3.93 ± 0.02^{ab}	3.85 ± 0.03^{b}	3.54 ± 0.11^{c}	3.39 ± 0.06^{c}
Lactose	5.12 ± 0.08^{e}	4.81 ± 0.06^{d}	4.39 ± 0.05^{c}	4.28 ± 0.03^{cd}	4.19 ± 0.04^{d}

BM, buffalo milk; CM, cow milk; MM, mixed milk (MM_1, mixed milk 1; MM_2, mixed milk 2; MM_3, mixed milk 3).
[a]Results represented as mean values with their standard deviation; means with different superscripts in rows differ significantly ($P < 0.05$).

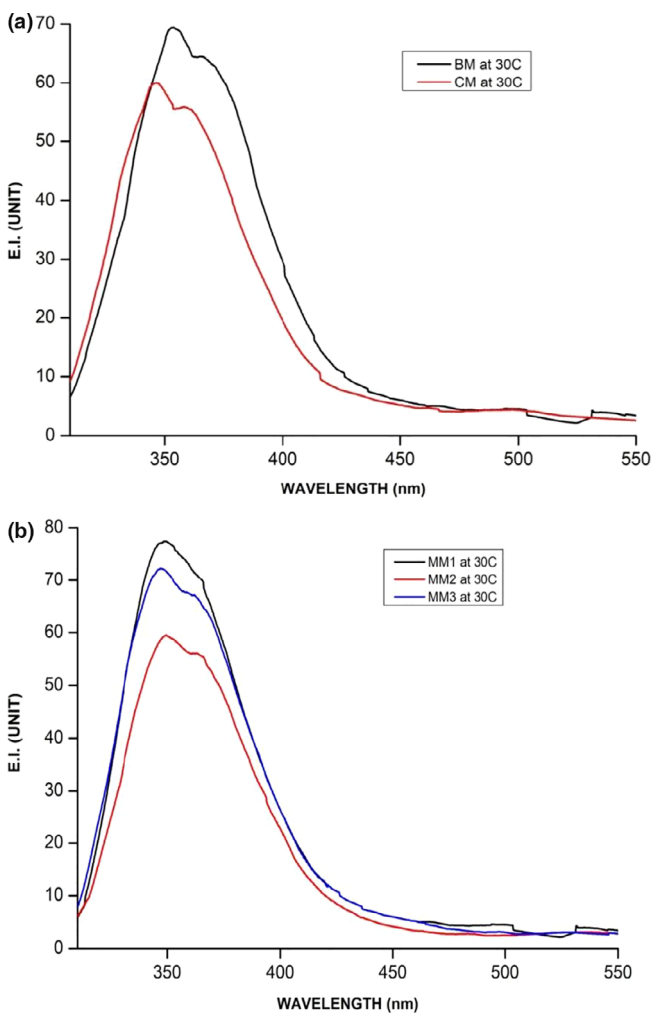

Figure 1 Tryptophan (Trp) emission intensity (E.I.) spectra at 30 °C of (a) raw CM and raw BM (black line – BM, red line – CM); (b) raw mixed milk samples (MM_1, MM_2, MM_3) (black line – MM_1, red line – MM_2, blue line – MM_3). [Colour figure can be viewed at wileyonlinelibrary.com]

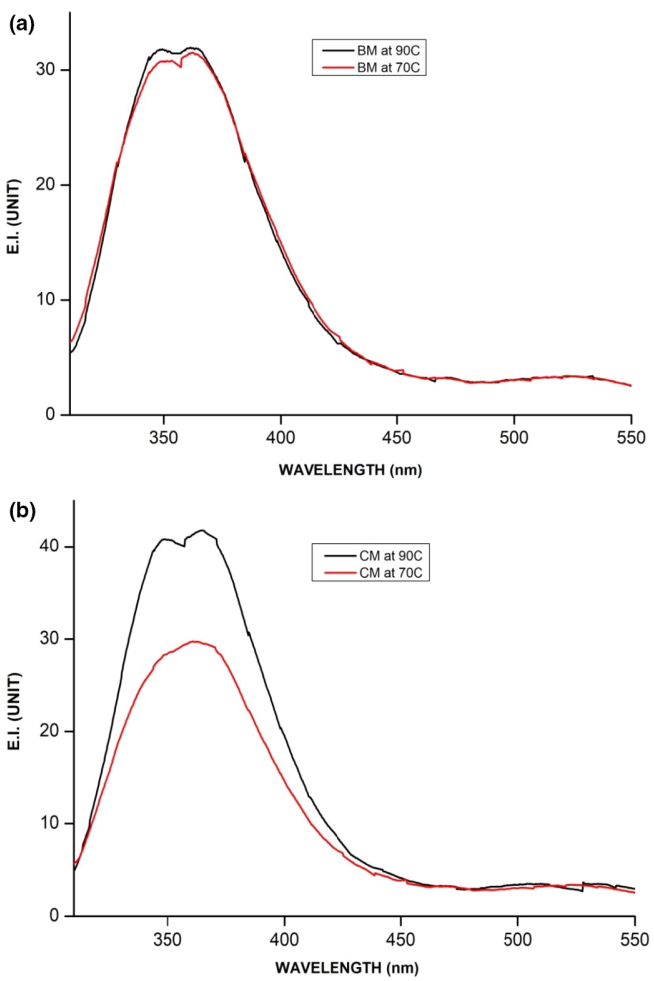

Figure 2 Tryptophan (Trp) emission spectra for the pre-heated (a) BM (black line – BM at 90 °C, red line – BM at 70 °C); (b) CM (black line – CM at 90 °C, red line – CM at 70 °C). [Colour figure can be viewed at wileyonlinelibrary.com]

milk at 30 °C, heated milk at 90 °C, pre-coagulated milk at 70 °C and coagulated milk with 0.15 mL of 1% citric acid). Milk samples were taken in a test tube followed by stirring to ensure homogeneity and were heated using a water bath and cooled down externally as the fluorescence instrument did not have the in-built thermostat facility. The sample from the test tube was transferred in the cuvette immediately

Table 3 Emission maxima and intensity values of the Trp residues in BM, MM and CM milk under different conditions.[a]

	BM		*MM_1*		*MM_2*		*MM_3*		*CM*	
	λ_{EM1} (nm)/ Emission intensity	*λ_{EM2} (nm)/ Emission intensity*	*λ_{EM1} (nm)/ Emission intensity*	*λ_{EM2} (nm)/ Emission intensity*	*λ_{EM1} (nm)/ Emission intensity*	*λ_{EM2} (nm)/ Emission intensity*	*λ_{EM1} (nm)/ Emission intensity*	*λ_{EM2} (nm)/ Emission intensity*	*λ_{EM1} (nm)/ Emission intensity*	*λ_{EM2} (nm)/ Emission intensity*
Raw milk at 30 °C	351/69	366/64.5	349/77.4	365/70.01	349.3/59.4	363.8/55.9	347.2/ 72.06	361.6/ 67.09	345/59.83	358/55.85
Heated milk at 90 °C	347/31.6	361/31.92	349.6/ 40.03	362.5/ 39.05	349.3/33.6	364.5/34.5	349.02/ 35.06	363.5/35.9	348/40.79	363/41.68
Milk cooled down at 70 °C	348/30.63	363/31.44	347.8/ 31.02	360.3/ 30.04	350.4/32.6	362.1/33.7	351.12/ 37.6	362.6/38.7	349/28.32	362/29.68
Milk at 70 °C with 1% citric acid	347/31.36	361/32.34	347.4/37.5	357.1/35.5	348.6/39.8	363.8/ 40.01	348.6/38.5	362.6/39.8	343/46.38	365/47.42

[a]λ_{EM1}, 1st Emission intensity (nm); λ_{EM2}, 2nd Emission intensity (nm); BM, buffalo milk; CM, cow milk; MM, mixed milk (MM_1, mixed milk 1; MM_2, mixed milk 2; MM_3, mixed milk 3).

for fluorescence measurement. pH and temperature were monitored for the sample in test tube in each step. Spectrofluorimeter (Hitachi F7000; Model-F–7000 FL) with solid state front-face accessory was used to record the emission spectra of all the milk samples under exactly identical conditions exciting tryptophan at λ_{ex} = 290 nm. 150 W Xenon light source (Hamamatsu Photonics, Japan) was used to generate the excitation light and it passed through 10 nm bandwidth interference grating system for wavelength fixation. A low scatter microcell was being used for measurements. All the measurements were replicated three times at each temperature condition to observe the repeatability, and best of these three was analysed further.

Statistical analysis

Data for raw milk analysis are presented as means ± standard error of the means. The variance analysis (ANOVA) was performed in Microsoft Office (Microsoft Excel, MS Office 7.0) to evaluate the significant variations in milk samples. Tukey's post hoc test was used to detect the significance of differences among the treatments at 95% confidence interval. Different superscripts were added to the values to elaborate the significant differences ($P < 0.05$).

RESULT AND DISCUSSION

Preliminary analysis of raw milk

Moisture content, total milk solids, milk protein, fat, lactose and SNF contents of all five raw milk samples are reported in Table 2. Data for protein and lactose contents indicate that they differ among species but to a smaller extent than fat content. Maximum total solids, fat and protein contents were present in BM (Table 2). The fat and casein content in mixed milk samples are reported to be higher as compared to CM. Moisture content was found to be more in CM sample. All the major nutrients are significantly higher in BM as compared to CM. Similar observations were reported by Wahid and Rosnina (2011). The changes in milk fat and protein percentage may be due to the changes in blood plasma lipids which also vary with animal's dietary changes (Bernabucci *et al*. 2015).

Coagulation studies through fluorescence

The whole experimental studies can be described through three phases: first phase at 30 °C; second phase when the milk is heated at 90 °C and cooled down to 70 °C; and the final third phase of acidification at 70 °C. Entire heat-induced protein denaturation (1st and 2nd phase) and acidic coagulation process (3rd phase) were monitored through fluorescence and described below.

Phase I – Raw milk samples at room temperature (30 ± 1 °C)

The raw CM and raw BM exhibited structured emission spectra when excited at 290 nm as Trp emission has two nearby isoenergetic transition states (Lakowicz 1999). Trp residues located in the apolar environment of casein micelles in raw milk would be unable to form any kind of H-bonds with the aqueous media (the continuous phase) and therefore exhibit structured emission (two emission maxima due to the two close isoenergetic states) for Trp residues. CM exhibited emission at 345 nm with a shoulder at 358 nm. Similar emission peaks are observed for BM observed at 350 nm and 366 nm but bathochromic-shifted

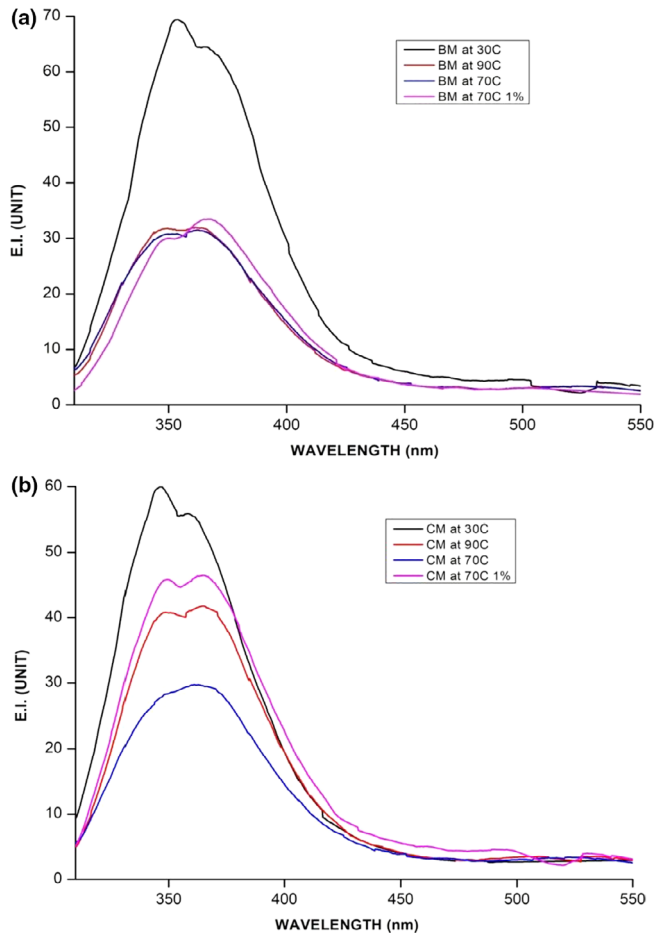

Figure 3 Tryptophan (Trp) emission spectra at different temperatures and with coagulant (1% of citric acid solution) (a) for BM (black line – BM at 30 °C, red line – BM at 90 °C, blue line – BM at 70 °C, pink line – BM at 70 °C with coagulant); (b) for CM (black line – CM at 30 °C, red line – CM at 90 °C, blue line – CM at 70 °C, pink line – CM at 70 °C with coagulant). [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 1a). This suggests that the Trp residues in BM are more exposed to the aqueous phase compared to the Trp residues in the CM. Milk protein is constituted of 76–86% casein and only 14–24% whey protein (Sindhu and Arora 2011). Therefore, emission of Trp residues from casein protein will dominate the emission of Trp residues from whey due to population difference.

In case of mixed milk, the spectral behaviour of Trp residues (Figure 1b) substantiates with the ratios in which CM and BM are mixed. MM_3 exhibited more of CM characteristics while MM_1 exhibits more of BM characteristics.

Phase II – Heated milk samples

Fluorescence intensity of Trp in milk decreased with increasing heat treatment (Dufour and Dufour and Riaublanc 1997; Birlouez-Aragon *et al*. 2002; Kulmyrzaev and Dufour 2002). In present study, both the milk samples (BM and CM) when heated at 90 °C, unfolding/conformational change of protein, possibly took place and Trp emission got highly quenched on interactions with other amino acid residues suggesting structural rearrangement of the protein molecules. The emission intensity values of the Trp residues clearly indicated that the quenching is much higher in BM compared to CM which possibly suggests that the extent of unfolding is more in BM than in CM at 90 °C (Figure 2a). As the Trp residues in BM caseins are more exposed to aqueous environment, it is quite natural that there is more probability of interaction of Trp moieties with other amino acid residues and hence more quenching of Trp emission in BM. Denaturation of β-LG takes place rapidly during heating and the denatured β-LG might possibly get attached on to the surface of the casein micelles. It is not clear if these interactions (between k-CN and whey proteins) unfold in micelles which further dissociate this complex into the serum, or if the complex is created in the serum itself (Singh 2004). This difference in the state and number of denatured protein content may be one of the reasons affecting the product yield and quality. The mixed milk samples also exhibited significant quenching upon heat treatment (Table 3) similar to CM and BM.

The structured emission spectra for CM completely disappeared while milk was cooled from 90 °C to 70 °C, whereas it remained to a certain extent for BM. These observations suggest that the Trp residues in each of the milk systems (preferably in CM) experience a homogeneous molecular environment on cooling to 70 °C. BM proteins did not show any significant change in emission intensity values as compared to CM proteins where there was further decrease of intensity (Figure 2b, Table 3). Buffering capacity of CM is less compared to BM due to low content of calcium phosphate thereby significant pH shift leads to instability in CM casein micelle structure. This kind of structural orientation possibly responsible for quenching the emission intensity of Trp residues in CM while almost no change in the emission intensity is observed for BM samples when milk was cooled from 90 °C to 70 °C.

Chandrapala *et al*. (2010) reported that alterations in pH and calcium activity on heating milk were largely reversible once the heated milk system was cooled in reconstituted skim milk solutions. This might be one of the reasons which affected the change in protein conformations due to partial or complete denaturation of β-LG and formation of denatured β-LG-k-CN complexes, leading to a change in emission intensity when the milk samples were cooled down. In case of our studies, it may be attributed to a reversible complex formation phenomenon upon acidification in cow milk sample (Figure 3). Similar results have been reported when solutions of β-LG reported both increasing and decreasing intensities with thermal treatment (Renard *et al*. 1998; Manderson *et al*. 1999; Elshereef *et al*. 2006). Concentration of

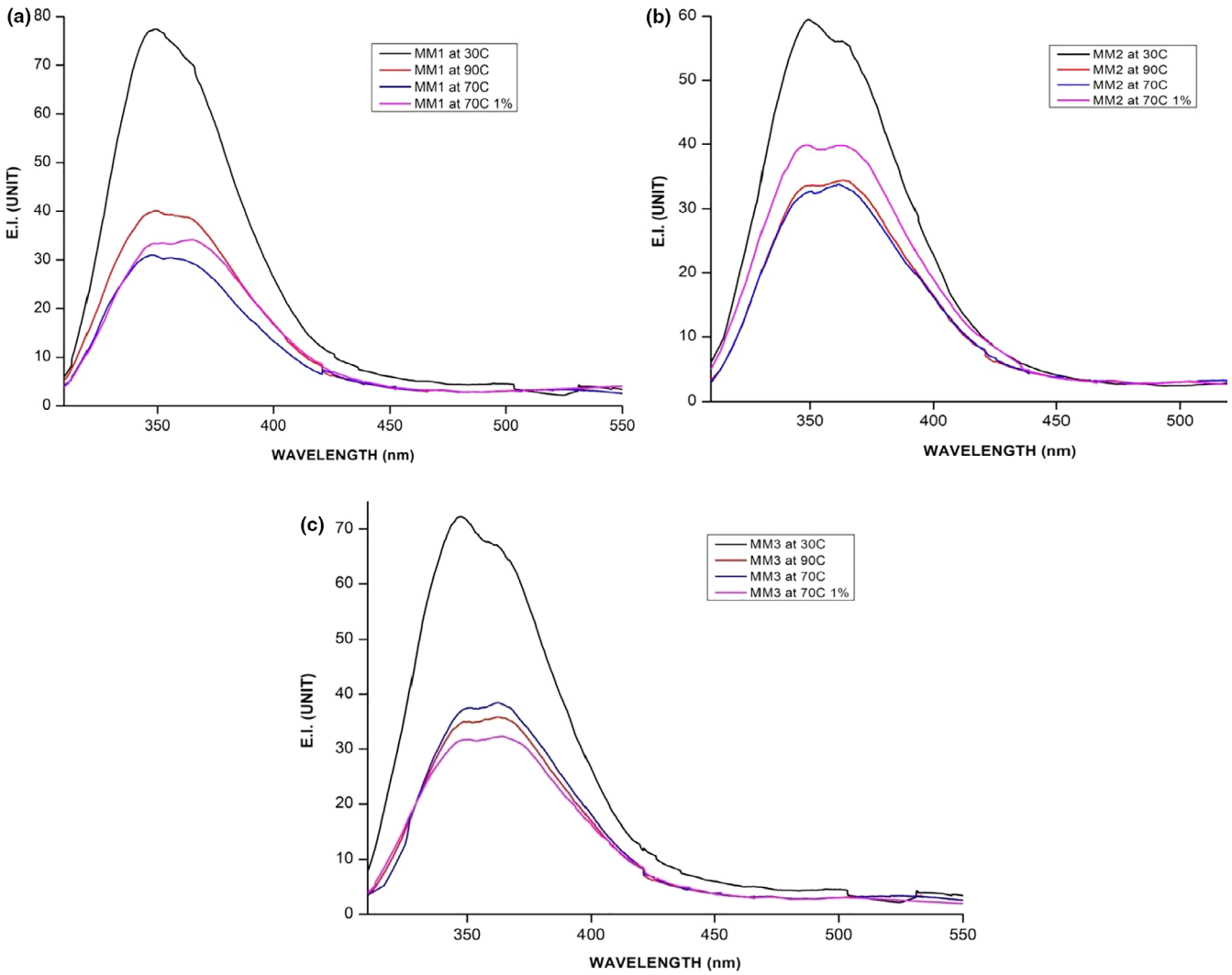

Figure 4 Tryptophan emission spectra at different temperatures and with coagulant (1% of citric acid solution) (a) for MM_1 (black line – MM_1 at 30 °C, red line – MM_1 at 90 °C, blue line – MM_1 at 70 °C, pink line – MM_1 at 70 °C with coagulant); (b) for MM_2 (black line – MM_2 at 30 °C, red line – MM_2 at 90 °C, blue line – MM_2 at 70 °C, pink line – MM_2 at 70 °C with coagulant); (c) for MM_3 (black line – MM_3 at 30 °C, red line – MM_3 at 90 °C, blue line – MM_3 at 70 °C, pink line – MM_3 at 70 °C with 1% citric acid coagulant). [Colour figure can be viewed at wileyonlinelibrary.com]

the fluorophores and phosphatase activity is influenced by the heat treatment in a similar pattern, as does the native β-LG content (Kulmyrzaev *et al*. 2005). Further scientific studies are needed to explain this reversible complex formation phenomenon on cooling of milk.

β-LG among other whey proteins (WP) contains more amount of Trp residues (58% of Trp in 147 mg Trp/g of total nitrogen in WP), upon heating/cooling the unfolding/conformational changes due to denaturation of β-LG to be taken into account(Delgado-Andrade *et al*. 2006; Huppertz *et al*. 2017; Kilara and Vaghela 2018). In CM containing mixed milk sample (MM_3), the β-LG content is high, and hence, its denaturation is also faster. Upon cooling, the bond between k-CN and β-LG is strengthened as the inorganic phosphate precipitates get dissolved slowly to regain the equilibrium leading to casein micelle stabilisation (Qian *et al*. 2017). This phenomenon changes the total protein equilibrium and alters the pH. In MM_1, the nature of the emission spectra and emission intensity remains unaltered (Table 3) possibly due to less β-LG content as compared to CM containing samples variation.

Phase III – Heat-acidinduced milk samples

Functionality of milk in relation with coagulum formation is significantly affected by heat treatment during acid coagulation of milk, thus affecting the heat-induced alterations in protein that have an impact on milk functionality and product quality (Chandrapala *et al*. 2010). During the acidification step on the onset of gelling, a slight decrease of the fluorescence intensity was observed at 350 nm in case of

BM. The emission intensity of Trp in CM showed a gradual change during the coagulation process as compared to BM. It might be due to the presence of Trp residues (casein micelles) present in the coagulum after coagulation in both the cases. Addition of citric acid (1%) to CM showed a significant bathochromic shift of the emission maxima to 365 nm supported by an increase in emission intensity. Not much significant change in the emission spectra (Figure 3) was observed under similar conditions for BM. It is to be noted that in the raw milk condition at 30 °C, the free Trp residues in BM were more exposed than the free Trp residues of CM. However, during heat-acid coagulation process, the aggregated Trp residues in β-LG-k-CN complexes of CM got more exposed to aqueous environment than the BM counterpart. The environment of the Trp residues became slightly less hydrophobic during pre-heating and cooling phases prior to coagulation for cow milk. No shift in the emission maxima for BM on addition of 1% citric acid was observed indicating that the Trp residues in the coagulated β-LG-k-CN complexes were not exposed the way CM caseins did. This observation well corroborates with the low hydration capacity of buffalo milk casein micelle with firm gel structure in comparison to cow milk casein micelle (Ahmad *et al*. 2008) which has more water retention capacity and less firmness in its gel structure. This difference in hydration capacity allows Trp residues of CM to reside in its more aqueous micellar core than the Trp residues of BM which has a less hydrated casein micelle core. Thus, the hydrophilic shift of Trp residues in CM in this phase can be attributed to the aqueous gel structure. It is to be noted that, on acidification at 70 °C, the Trp moieties regain their structured spectra to a certain extent in both, CM and BM. This could be due to the formation of the coagulum which restricts large number of interactions of the Trp moieties with the other residues. Thus the Trp residues experience quite a dynamic environment throughout the coagulation reaction.

Fagan *et al*. (2011) reported that during coagulation, the Trp emission intensity increased by 6.5% which could be an indicator to control the protein structural modifications and their physiochemical environment. From the above mentioned studies, it may be assumed that during coagulation mechanism, the coagulum contains more casein micelle-denatured whey protein assemblies with cluster of Trp residues attached. This might be a reason for increase in emission intensity value during acidic coagulation of CM at 70 °C.

In case of mixed milk samples, samples with higher BM percentage contain more protein. Thus the number of these assemblies (denatured whey proteins and casein micelles) increases with increase in CM content in mixed milk samples. Mixed milk samples with higher BM percentage (MM_1 and MM_2) had reduced emission intensity as compared to MM_3 as the whey protein content is more in CM (Table 3, Figure 4). Due to their complete denaturation and precipitation, the entire hydrophobic core comes in contact with the aqueous environment which leads to this decrease in intensity. Average denaturation transition intensity which signifies the stability of milk proteins upon heating was found to be maximum in case of MM_3 (75% CM, 25% BM) as compared to CM and BM, indicating the influence of β-LG and k-CN denaturation and further formation of β-LG-k-CN complex.

CONCLUSION

The results demonstrated that the heat-acid coagulation process of milk systems at the molecular level can be detected and analysed utilising fluorescence spectroscopy and tryptophan as a marker molecule. Different structural and conformational modifications during each phase change such as heating and acidification in milk proteins leading to coagulum formation were studied in this work. It was observed that the structured emission band of Trp reduced at a particular stage (heating), and again it regains its structure to a certain extent (acidification). Similarly, as far as the emission intensity of Trp moieties is concerned, there is a significant decrease in intensity followed by an increase. These behaviours of Trp molecules indicate that the moieties experience a series of different environmental conditions throughout the heat-acid coagulation process which points out that the heat-acid coagulation of milk systems is indeed a quite dynamic process. On the molecular level, it is concluded that due to heating, β-LG denatures and gets associated with micellar k-CN content which lead to pH shift and further aggravating the denaturation process on addition of acid. At different stages of coagulation, the environment of Trp residues varied but was comparatively hydrophilic for all milk samples following coagulation. The change in emission intensity and spectral value could be due to the dissociation of colloidal calcium phosphate and precipitation of micellar casein. The emission intensity enhancement and shift in all the milk samples were due to acidic coagulation which more significantly modified protein-component fluorescence properties than did the heating effect as micelle structural changes affect the protein folding/ unfolding phenomenon during acidification and coagulation. Thus fluorescence spectroscopic study can reveal the qualitative changes in the interaction of the proteins at molecular level upon heating and acidic coagulation for buffalo, cow and mixed milk systems. In future, possibly time-resolved fluorescence would throw more light on the dynamics of the heat-acid coagulation mechanism of different types of whole milk systems.

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AUTHOR CONTRIBUTIONS

Purba Chakraborty: Data curation; investigation; writing – original draft. **Bhaswati Bhattacharya:** Methodology; supervision; visualization. **Umashanker Shivhare:** Writing – review & editing. **Santanu Basu:** Conceptualization; methodology; project administration; resources; supervision; visualization; writing – review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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