CrossMark

ORIGINAL PAPER

A tool for explaining the differences on renneting characteristics of milks from different origins: the surface hydrophobicity approach

Seda Yildirim[1] · Yasar Kemal Erdem[1]

Abstract The differences between renneting characteristics of raw milk samples from different origins (bovine, ovine, caprine, buffalo) were investigated by protein surface hydrophobicity approach. 8-Anilinonaphthalene-1-sulfonic acid (ANS) binding method was used to evaluate surface hydrophobicity of raw milk samples and rennet precipitates. The following surface hydrophobicity parameters were calculated: number of surface hydrophobic sites (F_{max}), dissociation constant of the fluorescent ANS–protein complex (K_d), binding affinity of ANS to protein surface ($1/K_d$), the average tightness of binding of ANS to the protein (F_{max}/K_d), turnover number (k_{cat}), and protein surface hydrophobicity index (PSHI). The number of hydrophobic sites on the protein surface was found to be highest in cow milk, whereas ovine milk samples had the lowest number of hydrophobic sites and binding affinity to ANS. Protein content was not found directly related to the number of surface hydrophobic sites. The binding affinity of the proteins to ANS was greater in buffalo milk. PSHI was found to be the highest for bovine milk and the lowest for ovine milk. Renneting period was interpreted in two phases (enzymatic phase and flocculation phase) for each origin via ANS partition curves of rennet precipitates. Same trends between bovine–ovine and caprine–buffalo milks were observed during renneting. Buffalo milk completed both of two phases and total renneting period significantly earlier than the milks from the other origins. The hydrophobic parameters of proteins were found to play a key role on coagulation properties.

✉ Yasar Kemal Erdem
   erdem@hacettepe.edu.tr

   Seda Yildirim
   yildirims@hacettepe.edu.tr

[1] Department of Food Engineering, Faculty of Engineering, Hacettepe University, Beytepe Campus, 06800 Ankara, Turkey

INRA SCIENCE & IMPACT   Springer

1 Introduction

Since coagulation is the basis of cheese-making process, renneting properties of milk are thought to be the primary and the most significant factors affecting cheese yield and the resulting gel characteristics (Green and Morant 1981; Bonfatti et al. 2013). As well known, coagulation of milk by rennet is a consequence of destabilization of the casein micelles after proteolytic action of milk-clotting enzymes. Rennet-induced coagulation process is commonly divided into two phases: namely enzymatic phase and aggregation phase, although these stages are typically overlapped to some extent (Fox and McSweeney 1998). During the enzymatic phase, κ-casein which allows the stabilization of micelles is hydrolyzed by the enzyme at Phe_{105}-Met_{106} bond that results in the formation of caseinomacropeptide (CMP) and para-κ-casein (Walstra 1990).

Cow, sheep, goat, and buffalo milks, as well known, differ in general composition and physicochemical properties (Park et al. 2007). Sheep and buffalo milks are characterized with higher total solid, protein, and mineral content compared to cow and goat milks (Park et al. 2007; Ahmad et al. 2013). Although the economic quality criteria of goat and sheep milks are based on total protein concentration, caseins that are essential for cheese-making process should be considered as well. The structure of casein micelles in cow, goat, sheep, and buffalo milks was thought to be similar, but significant differences in micelle size have been reported between different origins (Brule et al. 2000). The main casein micelle fractions (α_{s1}-, α_{s2}- β-, and κ-caseins) in goat and sheep milks are similar to those in cow milk. However, it has been reported that the size and ratio of these fractions differ depending upon the origin. While the main α_s-casein fraction in bovine milk micelles is α_{s1}-casein, goat milk micelles lack in or have a low concentration of α_{s1}-casein, and unlike other small ruminants, dominated casein fraction in goat milk is β-casein (Jandal 1996; Feligini et al. 2009). Lower casein concentration, ratio of casein fractions, and relatively higher casein micelle size explain the weak texture of gels made from goat milk (Park et al. 2007)

The factors affecting coagulation properties have been studied by several authors in bovine milk, less so in ovine milk, and, to lesser extent, in caprine and buffalo milks (Remeuf et al. 1989; Remeuf et al. 1991; Ramos and Juarez 2003). Raynal and Remeuf (1998) and Raynal-Ljutovac et al. (2008) reported that the colloidal stability of sheep and goat milks is different from that of cow milk, thus leading to a different coagulation behavior which has lower gelation time than that of cow milk. On the other hand, Grandison (1986) explained the faster coagulation of sheep milk with high casein and protein content. Goat milk is characterized by a short flocculation time and lower firmness of resulting gel. Calvo (2002) evaluated the rennet coagulation period of bovine, ovine, and caprine milks and reported that enzymatic phase proceeds faster in bovine milk than ovine and caprine milks, whereas the difference between caprine and ovine milk samples is insignificant. In addition, aggregation rate was found to be higher in caprine and ovine milk compared to cow milk. Although few studies carried out for buffalo milk, it has been concluded that buffalo milk has higher coagulation rate than cow milk (Abd El-Salam and El-Shibiny 2011).

Protein surface hydrophobicity is accepted to be one of the most important factors affecting technical and functional properties of proteins such as colloidal stability, solvation, hydration, and denaturation (Kato and Nakai 1980; Hiller and Lorenzen 2008). Caseins are

characterized by their hydrophobicity, relatively high charge, and many proline and few cysteine residues (Walstra 1990). Since the hydrophobic residues are rather exposed, caseins have much tendency to interact with other casein micelles or proteins and also some ligands according to the hydrophobic character of the micelle (Fox and McSweeney 1998). Surface hydrophobicity of casein micelles could give some information about micelle structure (Erdem 2000). One of the most common methods used for evaluation of the protein surface hydrophobicity is titrating protein solutions with increasing concentrations of a fluorescent probe, among which ANS is much preferred for food proteins. This approach provides useful information about the number and binding affinity of hydrophobic sites.

The results of previous studies related to rennet-induced coagulation properties of milks from different species were commonly interpreted with protein and casein content solely. However, besides quantitative properties, qualitative properties such as distance between casein micelles and hydrophobic characteristics are thought to be important especially during flocculation and aggregation stage. The aim of this study was to explain the different renneting characteristics of milks from different origins with surface hydrophobic properties.

2 Materials and methods

2.1 Milk samples

Raw bovine (Holstein), ovine (Kıvırcık), caprine (Saanen), and buffalo (Anatolian water buffalo) milk samples were obtained during lactation period from a domestic dairy (A.O.C., Ankara). All samples were defatted by centrifuging at 3000 rpm and 7 °C for 10 min (Sigma 3-30K, UK). Skimmed milk samples were preserved by addition of sodium azide (1:10,000, *w/v*) and stored at 4 °C.

2.2 General analyses

Fat content of the samples was determined by the Gerber method (IDF 1981). Determination of total solid content was carried out according to the IDF standard (IDF 1982). The pH was measured with a pH meter (Sartorius, TE214S, Germany). Titratable acidity (°SH) of samples was established by the Soxhlet–Henkel method (AOAC 1995). Gravimetric method was carried out in order to determine ash content of all samples (AOAC 1990). Protein contents of samples were determined according to Bradford (1976). In order to determine any differences in protein profile of milks from different origins, urea PAGE was carried out according to Andrews (1987).

Additionally, the protein profile of the samples was analyzed by reverse phase (RP)-HPLC. A complete Agilent 1100 series of the chromatographic system (Agilent Tech, USA) equipped with a quaternary pump, an autosampler, a column thermostat, a degasser, and a UV detector was used. All data was treated by ChemStation software. The separation was performed on a RP analytical column ACE C8 (300 Å, 5 μm, 250 L, 4.6 ID). The linear gradient elution was performed as follows: from 20 to 40% solvent B in 6 min, from 40 to 45% solvent B in 10 min, from 45 to 50% solvent B in 3 min, followed by an isocratic elution at 50% solvent B during 4 min, then from 50 to 70% solvent B in 3 min, from 70 to 100% solvent B in 1 min, kept constant at 100%

INRA
SCIENCE & IMPACT

Springer

solvent B for 1 min, followed by an equalization to 20% solvent B in 5 min. Elution was carried out at a flow rate of 1 mL.min^{-1} using two solvents: solvent A containing 0.1% (v/v) trifluoroacetic acid (TFA) in acetonitrile–water (10:90, v/v) and solvent B containing 0.1% (v/v) TFA in acetonitrile–water (90:10, v/v). The column temperature was kept at 40 °C, and the elution was monitored at 220 nm. Standard purified proteins including α-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin A, and β-lactoglobulin B (Sigma, Germany) were used after appropriate dilution for identifying milk proteins in samples.

2.3 Determination of protein surface hydrophobicity of milk samples

ANS binding method was used due to the complex formation between ANS and proteins via hydrophobic bindings. Although some studies claimed that using a non-ionic probe instead of ANS could be a better option, it has been widely accepted that at non-alkaline pH, ANS is less charged and interacts with proteins mainly by hydrophobic attractions rather than ionic (Hawe et al. 2008). Protein surface hydrophobicity of milk samples was determined according to Erdem (2000). The relative fluorescence intensity of the milk samples from different origins was measured by using a spectrophotometer (Varian Cary Eclipse Fluorescence Spectrophotometer, USA) with a typical glass cell. 8-Anilinonaphthalene-1-sulfonic acid (ANS) was used as the fluorescence probe for this study, and the measurements were carried out at λ_{ex}=390 nm and λ_{em}= 480 nm. The fluorescence intensity of the samples without ANS was measured as blank in order to eliminate turbidity effect. Kinetic parameters (F_{max}, K_d, and k_{cat}) were calculated as the averages of four different kinetic approaches: Lineweaver–Burk, Hanes–Woolf, Eadie–Hofstee, and Michaelis–Menten via a software developed by one of our colleagues. Protein surface hydrophobicity index (PSHI) was determined after the samples were titrated with increasing concentrations of ANS (final concentration of ANS in milk samples varied from 0 to 200 μmol.L^{-1}) according to Erdem (2000) with the following equation ([P]: protein concentration).

$$PSHI = \frac{F\text{max}}{(K\text{d} * [P])} \tag{1}$$

2.4 Coagulation experiment

Coagulation of milk samples was carried out according to Erdem (2000). Coagulation procedure was performed as follows: ANS was added to 50-mL milk samples with a final concentration of 0.2 mmol.L^{-1} in an ice bath, and after 5 min of equalization, 100 μL rennet (5%, v/v, Maxiren 600 IMCU) was added. It was observed that 90% of ANS was bonded in first 5 min after addition if the concentration is high enough (Data not shown). Immediately after adding rennet, milk samples were divided into 5 mL aliquots and placed in a water bath at 35 °C. Aliquots were removed from the water bath at certain time intervals (0, 1, 3, 5, 10, 15, 20, 30, 60, 90 min) exceeding the flocculation time determined by the Berridge method (Berridge 1952) and placed into the ice bath in order to stop the enzymatic reaction. Then, all aliquots were centrifuged at 10,000×g and 4 °C for 20 min. With a volume equal to the removed supernatant, 8 mol.L^{-1} urea was added to precipitates obtained by centrifugation and kept overnight

at 10 °C. ANS partition in rennet precipitates was determined as follows: 100 μL of precipitate solutions of each tube was made up to 5 mL with Triton X-100 (5%, w/v, Sigma, Germany), and fluorescence intensities were measured before and after adding 10 μL 1 mmol.L^{-1} ANS into the tubes. The amount of ANS that bound to the proteins in rennet precipitates was calculated by appropriate equations. Protein partition of the precipitates was determined by the Bradford (1976) method. Curves obtained by ANS partition were used separately in order to evaluate renneting period for all milk samples. Change in protein profile of the precipitates during renneting period was also assessed by urea PAGE and RP-HPLC methods as described in Sect. 2.2.

2.5 Statistical analyses

All the experiments were carried out for at least four times. The means with the standard deviation (SD) were calculated based on the overall data collected throughout the survey. Analysis of variance (ANOVA) was carried out to determine the significance at a confidence level of 95% by using SPSS for Windows (IBM, SPSS Inc., Chicago, IL, USA).

3 Results

3.1 General composition

General composition of skimmed milk samples is shown in Table 1. The difference between pH values of milk samples from different origins was insignificant ($p>0.05$). Titratable acidity values were found to be the highest for sheep milk whereas the lowest for the goat milk. Total solid and protein contents of sheep and buffalo milks were found to be significantly higher ($p<0.05$) than those of cow and goat milks, as expected. Average values of protein content were 35.5±3.10 g.L^{-1}, 40.6±3.60 g.L^{-1}, 54±4.30 g.L^{-1}, and 47.7±2.00 g.L^{-1} for cow, goat, sheep, and buffalo milks, respectively. The general composition of milk samples is representative for different origins (Park et al. 2007; Ahmad et al. 2013).

Table 1 Comparison of general composition of cow, goat, sheep, and buffalo milks

	Cow	Goat	Sheep	Buffalo
pH	6.64±0.06	6.64±0.07	6.63±0.04	6.61±0.06
Titratable acidity (SH)	6.98±0.41	6.45±0.66	8.88±0.28	7.84±0.68
Solid non-fat (%)	8.74±0.34	9.27±0.50	12±0.50	10.8±0.84
Ash (%)	0.748±0.03	0.809±0.04	0.925±0.04	0.787±0.06
Fat (%)	0.05±0.05	0.25±0.10	0.1±0.05	0.1±0.05
Protein (g.L^{-1})	35.5±3.10	40.6±3.60	54±4.30	47.7±2.03

Values are mean±SD for four replicates

3.2 Protein surface hydrophobicity

Figure 1 shows ANS titration curves that were obtained by relative fluorescent intensities of milk samples from each origin. The fluorescence intensity of milk samples which were titrated with increasing amount of ANS increased until a maximum value and then kept nearly constant. The slope of the curves showed significant differences among different origins. The slope of cow milk samples was found to be highest whereas sheep milk samples had the lowest value. The differences in the ANS titration curve slopes within different origins were in accordance with Yuksel et al. (2012). The differences between kinetic parameters that were calculated for cow, goat, sheep, and buffalo milks based upon ANS titration curves are shown in Fig. 2. F_{max} represents both the maximum fluorescence that could be attained at given conditions and also the maximum number of surface hydrophobic sites on the protein (casein micelle) surface. $1/K_d$ represents the binding affinity of protein to ANS. F_{max}/K_d gives the tightness of protein–probe (ANS) binding. Protein surface hydrophobicity index (PSHI) denotes the average tightness of ANS binding per protein and represents the overall surface hydrophobicity. F_{max} value was found to be highest for cow milk and followed by goat, buffalo, and sheep milks, respectively. It was observed that the difference between F_{max} values of samples from different origins was significant ($p<0.05$). Although the number of hydrophobic sites was not found to be highest in buffalo milk, the affinity of the proteins to the probe ($1/K_d$) was highest for buffalo milk. The difference between $1/K_d$ values among different origins was found to be significant ($p<0.05$) except the difference between cow and goat milk samples ($p>0.05$). Additionally, cow milk had the highest tightness of "ANS–protein" binding (F_{max}/K_d). $F_{max}/[P]$ values that represent the number of hydrophobic sites of binding per protein were calculated for each origin in order to overcome the effect of protein content. Surprisingly, the number of surface hydrophobic sites per protein showed the same response as in the F_{max} values. The highest F_{max}/P value was observed in cow milk followed by goat, buffalo, and sheep milks, respectively. Protein surface hydrophobicity index (PSHI) denotes an overall surface hydrophobicity for milk proteins.
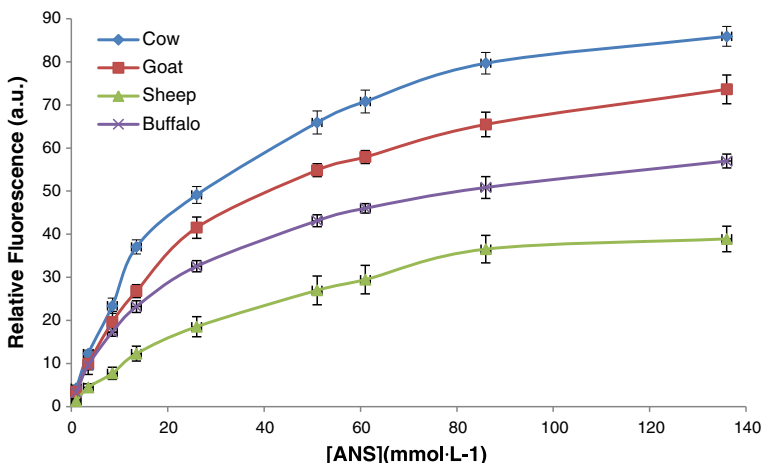

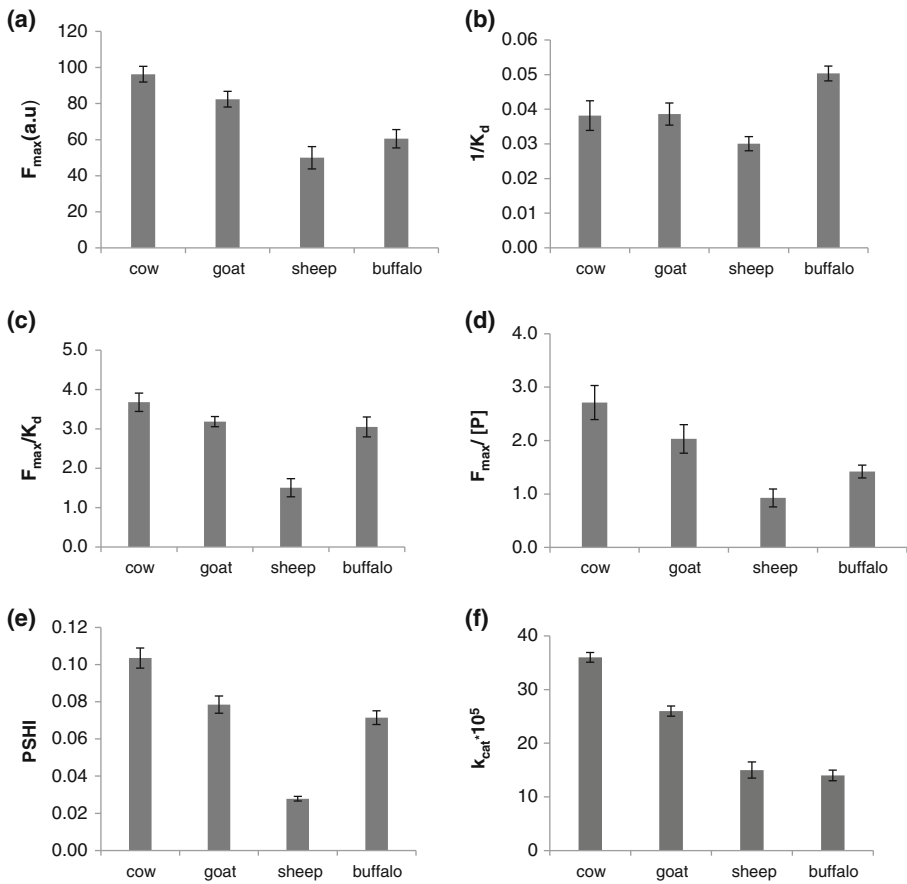Fig. 1 ANS titration curves of skimmed milk samples from different origins (cow, goat, sheep, and buffalo)

Fig. 2 Kinetic parameters of cow, goat, sheep, and buffalo milk samples showing number of surface hydrophobic sites (a), ANS binding affinity to protein (b), tightness of ANS binding to protein (c), number of surface hydrophobic sites per protein (d), protein surface hydrophobicity index (e), and turnover number (f)

PSHI of cow milk was found to be greater and followed by goat, buffalo, and sheep milks, respectively. Except goat and buffalo milks ($p>0.05$), PSHI values were significantly different for milks from different origins ($p<0.05$).

Sheep milk had the lowest parametric values in hydrophobicity approach. The number of surface hydrophobic sites was found to be the lowest despite their high protein content. Furthermore, the affinity, tightness, and PSHI of the surface hydrophobic interactions were found to be lowest as well for sheep milk than those of other origins.

3.3 Changes in protein surface hydrophobicity during renneting

During the rennet coagulation of milks, ANS concentrations, which bound to the rennet precipitates, were calculated, and results are shown in Fig. 3. It can be apparently seen from the figure that similar trends were observed between cow–sheep milks and goat–buffalo milks during the renneting period. ANS partition curves were evaluated separately for each origin, and parametric values representing the time required for
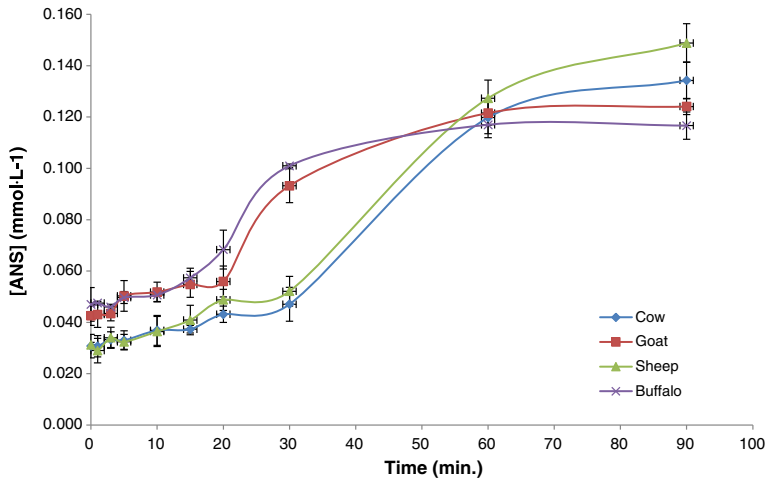
Fig. 3 ANS concentrations that bound to proteins in rennet precipitates over renneting time for cow, goat, sheep, and buffalo milk samples

completion of the two phases were calculated (Table 2). The lag phase, where there was no significant change in bound ANS concentration, was considered as enzymatic phase (EP), and the logarithmic phase was referred to the flocculation phase (FP).

It was observed that renneting time of buffalo milk was significantly shorter compared to other origins ($p<0.05$) and followed by goat, sheep, and cow milks, respectively. Likewise, when renneting period was discussed in two stages, buffalo milk completed both phases earlier than other evaluated milks. Buffalo and goat milks were found to complete the first stage (EP) significantly faster than cow and sheep milk samples. Additionally, the difference between EP durations for cow and sheep milks was not significant while these origins showed a greater flocculation time than that of goat and buffalo milks. There were no significant differences in flocculation time of cow and sheep milks. Similar results were obtained when protein partition curves were evaluated over renneting time (data not shown).

3.4 Changes in protein profile of different origins during renneting

Figure 4 shows the urea PAGE electrophoretograms obtained from rennet precipitates from different origins during renneting period. It can be seen from the urea PAGE gels that β-casein concentration is significantly higher in goat milk, as expected. Dramatic changes in concentration of casein fractions for bovine and ovine milk samples were observed between line 7 and line 8, meaning that the majority of casein micelles flocculated and gathered at a time near to 60′ and resulted in a high casein concentration

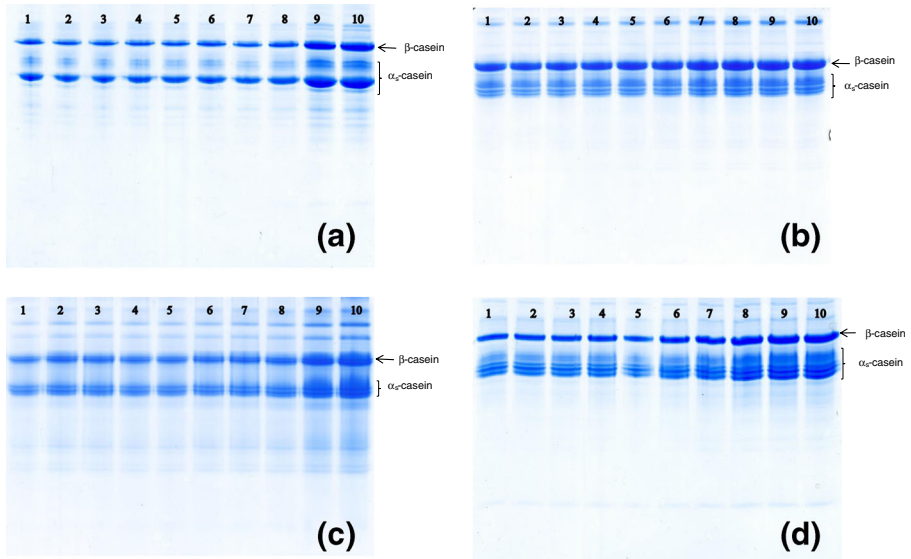|	Enzymatic phase (EP) (min)	Flocculation phase (FP) (min)	Renneting time (RT) (min)
Table 2 Parametric values of time periods calculated from bound ANS concentration curves of cow, goat, sheep, and buffalo milk samples			
Cow	24.5	50.0	74.5
Goat	18.1	44.3	62.4
Sheep	26.5	44.9	71.4
Buffalo	8.5	34.0	42.5

INRA
SCIENCE & IMPACT

Fig. 4 Urea PAGE electrophoretograms during renneting period for **a** bovine, **b** caprine, **c** ovine, and **d** buffalo milks at certain time intervals (*line 1*, 0 min; *line 2*, 1 min; *line 3*, 5 min; *line 4*, 10 min; *line 5*, 15 min; *line 6*, 20 min; *line 7*, 30 min; *line 8*, 60 min; *line 9*, 90 min)

in the precipitates. However, changes in buffalo and goat milk samples were different from those in bovine and ovine milks. β-Casein concentration of goat milk was increased nearly constantly during renneting period, and a similar trend was observed for buffalo milk with a notable increase after 60′.

Protein profile of milk samples and rennet precipitates which were obtained during the renneting period was also investigated with RP-HPLC. Since κ-casein peaks were relatively smaller and might cause increased experimental error, only α-casein and β-casein peaks were evaluated. During renneting period, changes in α_s-casein and β-casein concentrations calculated from peak areas in precipitates are shown in Fig. 5.

According to Fig. 5a, FP was completed nearly in 50 min for buffalo milk whereas samples from other analyzed origins completed FP after 70 min. At the end of renneting period, sheep milk had the highest α_s-casein content. The time duration where FP progressed showed similar trends when β-casein concentrations were evaluated (Fig. 5b) for different origins. However, goat milk, for which β-casein concentration continually increased, differed in the way followed during flocculation. The results obtained by RP-HPLC were in accordance with gel electrophoresis, in general.

4 Discussion

In common, the protein content of milk is thought to be the primary factor affecting the coagulation process of milk and the properties of resultant products (Jõudu et al. 2008). It is widely accepted that protein–protein bindings are established during the flocculation process of para-κ-casein micelles, occurred hereafter a given extent of κ-casein hydrolysis. Hydrophobic bindings, which were related to the surface hydrophobicity of para-κ-casein micelles, are thought to be one of the main attractive forces which control
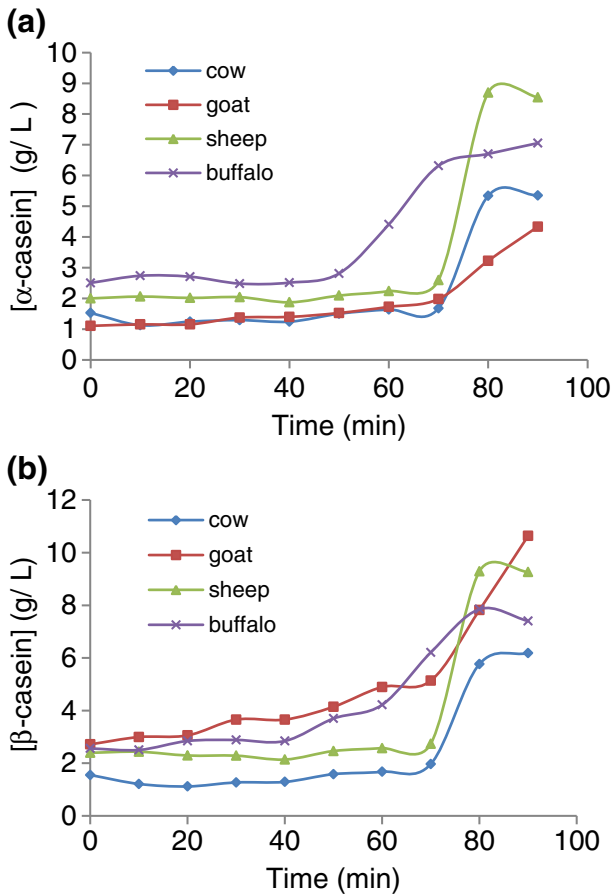
(a)

(b)

Fig. 5 Changes in α-casein (**a**) and β-casein (**b**) concentrations of rennet precipitates obtained during renneting period

the gel formation during renneting. According to this approach, it is argued in the present study that the number of surface hydrophobic sites of the casein micelles and their affinity might determine the renneting characteristics of milks.

The protein content and surface hydrophobicity of the milk samples from different origins were found to be different as expected. However, the differences in protein content could not explain the differences in surface hydrophobic sites. It was observed that the higher casein content did not result in higher surface hydrophobicity. The number of surface hydrophobic sites may contribute to different micelle structure and may have an impact on renneting characteristics. It is argued that more compact micelle structure causes surface hydrophobic sites buried inside the micelles. According to the surface hydrophobicity approach, it was suggested that sheep milk had a more compact micellar structure due to having the highest content of casein but the lowest number of surface hydrophobic sites in accordance with the results obtained by Yuksel et al. (2012).

The renneting characteristics were studied by several authors mostly for cow, goat, and sheep milks and, to a lesser extent, for buffalo milk (Remeuf et al. 1989; Remeuf et al. 1991; Bonfatti et al. 2013). However, most of the studies dealing with different

renneting behaviors of milks from different origins were based on the rheological measurements, and these differences were explained by protein contents of milks. The coagulation patterns of the milk samples from different origins were found interesting in this study. It was observed that there were two different patterns which represent the main two stages of renneting. However, interpreting the results via solely surface hydrophobicity parameters may not explain the differences and similarities between renneting characteristics. The collision distance of proteins, micelle size, micelle integrity, etc. should also be considered as the factors affecting the coagulation process (Walstra 1990; Mellema et al. 1999). The similarity observed for the sheep and cow milks by terms of surface hydrophobicity could be possibly explained as follows: sheep milk had the highest casein content which causes a shorter collision distance between micelles during aggregation phase, leading to an expectation that sheep milk might have a shorter aggregation period when compared to cow milk. However, our results showed a similar aggregation time of para-κ-casein micelles for cow and sheep milks. It was thought that the lowest hydrophobic properties (number of surface hydrophobic sites, binding affinity, and PSHI) of sheep milk could be responsible for the longer aggregation and renneting time than expected. On the other hand, cow milk contains lower casein but higher surface hydrophobic sites than sheep milk. The more surface hydrophobic sites, higher PSHI, and aggregation rate (k_{cat}) might overcome the longer collision distance, a consequence of lower casein content, of cow milk. Our findings of EP and FP durations for cow, goat, and sheep milks are in accordance with the results obtained by Raynal and Remeuf (1998) and Raynal-Ljutovac et al. (2008) who found that cow milk has a longer renneting time than sheep and goat milks. However, they found no significant differences between coagulation time of goat and sheep milks, whereas our findings showed that goat milk samples have shorter coagulation time when compared to sheep milk. On the other hand, Calvo (2002) concluded that the EP of coagulation progresses faster for cow milk than for sheep milk.

The relation between surface hydrophobicity differences and renneting time was mostly attributed to casein micelle structure, but it could be speculative because of the lack of the additional data for casein micelles in this study.

The fastest renneting and flocculation process was observed for buffalo milk that followed a very similar pattern with goat milk. However, the abovementioned explanation for cow and sheep milk samples could not explain the similar renneting tendency of goat and buffalo milks. While the casein content was almost the same for both of them, the hydrophobicity of casein micelles was found to be different. Although the number of surface hydrophobic sites was higher in goat milk, the affinity of the surface hydrophobic sites of the buffalo milk was found to be highest. On the other hand, the binding affinity, tightness of binding, and PSHI were similar for buffalo and goat milk samples. These similarities may explain the similar renneting pattern of these milks. However, the apparent short renneting time of buffalo milk could be explained by having a greater casein micelle size (Ahmad et al. 2013) which may contribute to the short flocculation time due to shorter collision distance between micelles (Bonomi et al. 1988).

5 Conclusion

This study indicates some differences in renneting kinetics of different-originated milk samples with milk protein surface hydrophobicity approach. The earlier studies carried

out to determine the differences on renneting characteristics of bovine, ovine, and caprine milks were mostly explained by casein content and micelle size variations. However, at aggregation stage of renneting, hydrophobic bonds formed between para-κ-casein micelles as well as other attractions; thus, surface hydrophobicity of proteins is assumed to be an important factor for gel formation. To our knowledge, this is the first study which explains some of the renneting characteristics and differences between origins via protein surface hydrophobicity. It was found that protein content is not directly related to the number of surface hydrophobic sites. Besides the protein content, hydrophobic properties are important factors affecting the renneting period. This approach would be useful and contributing to the renneting researches. The interesting results of renneting performance of buffalo milk may be useful for the local manufacturers in order to optimize the cheese making process. Besides, the findings on the similarity between bovine–ovine and caprine–buffalo milk during the renneting may be used to optimize the cheese manufacturing parameters with the combination of the origins which showed similar coagulation patterns.

The results obtained point to the importance of knowledge about hydrophobic characteristics of casein micelles during rennet-induced coagulation in order to assess the process in detail. It is suggested that renneting period should be monitored by rheological measurements and micelle structures of the different milks have to be discussed by appropriate methods.

References

Abd El-Salam MHA, El-Shibiny S (2011) A comprehensive review on the composition and properties of buffalo milk. Dairy Sci Technol 91:663–699

Ahmad S, Anjum FM, Huma N, Sameen A, Zahoor T (2013) Composition and physico-chemical characteristics of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and vitamins. J Anim Plant Sci 23:62–74

Andrews AT (1987) Electrophoresis: theory, techniques and biochemical and clinical applications. Oxford University Press, New York

AOAC (1990) Ash of milk (Gravimetric method) No: 945.46. Official methods of analysis of Association of Official Analytical Chemists. AOAC, Virginia, USA

AOAC (1995) Acidity of milk, titrimetric method No: 947.05. Official methods of analysis of Association of Official Analytical Chemists. AOAC, Virginia, USA

Berridge NJ (1952) An improved method of observing the clotting of milk containing rennin. J Dairy Res 19: 328–332

Bonfatti V, Giantin M, Rostellato R, Dacasto M, Carnier P (2013) Separation and quantification of water buffalo milk protein fractions and genetic variants by RP-HPLC. Food Chem 136:364–367

Bonomi F, Iametti S, Pagliarini E, Peri E (1988) A spectro-fluorimetric approach to estimation of the surface hydrophobicity modifications in milk proteins upon thermal treatment. Milchwissenschaft 43:281–285

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brule G, Lenoir J, Remeuf F (2000) In: Eck A, Gillis JC (eds) The casein micelle and milk coagulation. Lavoisier Publishing, Paris

Calvo MM (2002) Influence of fat, heat treatments and species on milk rennet clotting properties and glycomacropeptide formation. Eur Food Res Technol 214:182–185

Erdem YK (2000) Influence of ultrafiltration on modification of surface hydrophobic sites of the milk protein system in the course of renneting. J Food Eng 44:63–70

Feligini M, Bonizzi I, Buffoni JN, Cosenza G, Ramunno L (2009) Identification and quantification of $\alpha s1$, $\alpha s2$, β and κ-caseins in water buffalo milk by reverse phase-high performance liquid chromatography and mass spectrometry. J Agric Food Chem 57:2988–2992

Fox PF, McSweeney PLH (1998) Dairy chemistry and biochemistry. Blackie Academic & Professional, London

Grandison A (1986) Causes of variation in milk composition and their effects on coagulation and cheese making. Dairy Ind Int 51:21–24

Green ML, Morant SV (1981) Mechanism of aggregation of casein micelles in rennet treated milk. J Dairy Res 48:57–63

Hawe A, Sutter M, Jiskoot W (2008) Extrinsic fluorescent dyes as tools for protein characterization. Pharmaceut Res 25(7):1487–1499

Hiller B, Lorenzen PC (2008) Surface hydrophobicity of physicochemically and enzymatically treated milk proteins in relation to techno-functional properties. J Agric Food Chem 56:461–468

IDF (1981) Determination of fat contents, gerber butyrometers. Int Stand FIL-IDF 105:1981

IDF (1982) Milk and milk products—determination of total solids content. Int Stand FIL-IDF 21A:1982

Jandal JM (1996) Comperative aspects of goat and sheep milk. Small Ruminant Res 22:177–185

Jõudu I, Henno M, Kaart T, Püssa T, Kärt O (2008) The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. Int Dairy J 18:964–967

Kato A, Nakai S (1980) Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties. Biochim Biophys Acta 624:13–20

Mellema M, Leermakers FAM, de Kruif CG (1999) Molecular mechanism of the renneting process of casein micelles in skim milk, examined by viscosity and light-scattering experiments and simulated by model SCF calculations. Langmuir 15:6304–6313

Park YW, Juarez M, Ramos M, Haenlein GFW (2007) Physico-chemical characteristics of goat and sheep milk. Small Ruminant Res 68:88–113

Ramos M, Juarez M (2003) In: Roginski H, Fuquay JW, Fox PF (eds) Sheep milk. Academic Press, Amsterdam

Raynal K, Remeuf F (1998) The effect of heating on physicochemical and renneting properties of milk: a comparison between caprine, ovine and bovine milk. Int Dairy J 8:695–706

Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliar Y (2008) Composition of goat and sheep milk products: an update. Small Ruminant Res 79:57–72

Remeuf F, Lenoir J, Duby C (1989) A study of the relations between physicochemical characteristics of goat milks and their renneting properties. Lait 69:499–518

Remeuf F, Cossin V, Dervin C, Lenoir J, Tomassone R (1991) Relationships between physicochemical characteristics of milks and their cheesemaking properties. Lait 71:397–421

Walstra P (1990) On the stability of casein micelles. J Dairy Sci 73:1965–1979

Yuksel Z, Avcı E, Uymaz B, Erdem YK (2012) General composition and protein surface hydrophobicity of goat, sheep and cow milk in the region of Mount Ida. Small Ruminant Res 106:137–144