J. Dairy Sci. 96:4182–4190
http://dx.doi.org/10.3168/jds.2012-6333

Protein composition affects variation in coagulation properties of buffalo milk

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ABSTRACT

The aim of this study was to investigate the effects exerted by the content of casein and whey protein fractions on variation of pH, rennet-coagulation time (RCT), curd-firming time (K_{20}), and curd firmness of Mediterranean buffalo individual milk. Measures of milk protein composition and assessment of genotypes at *CSN1S1* and *CSN3* were obtained by reversed-phase HPLC analysis of 621 individual milk samples. Increased content of α_{S1}-casein (CN) was associated with delayed coagulation onset and increased K_{20}, whereas average pH, RCT, and K_{20} decreased when β-CN content increased. Milk with low κ-CN content exhibited low pH and RCT relative to milk with high content of κ-CN. Increased content of glycosylated κ-CN was associated with unfavorable effects on RCT. Effects of milk protein composition on curd firmness were less important than those on pH, RCT, and K_{20}. Likely, this occurred as a consequence of the very short RCT of buffalo milk, which guaranteed a complete strengthening of the curd even in the restricted 31 min time of analysis of coagulation properties and for samples initially showing soft curds. Effects of *CSN1S1-CSN3* genotypes on coagulation properties were not to be entirely ascribed to existing variation in milk protein composition associated with polymorphisms at *CSN1S1* and *CSN3* genes. Although the role of detailed milk protein composition in variation of cheese yield needs to be further investigated, findings of this study suggest that modification of the relative content of specific CN fractions can relevantly influence the behavior of buffalo milk during processing.
Key words: buffalo, milk coagulation, casein, whey protein

INTRODUCTION

Renneting properties of milk are crucial aspects because coagulation is the basis of the cheese-making process. Although several factors affecting renneting properties of buffalo milk have been investigated (Potena et al., 2001; Ariota et al., 2007; Cecchinato et al., 2012), knowledge of the major sources of variation of buffalo milk coagulation properties (**BMCP**) is still scarce. The effects of the composition of the 6 major milk proteins (α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN, together with the 2 whey proteins α-LA and β-LG) on BMCP have never been investigated in detail, although different κ-CN patterns seem to be responsible for variation of coagulation time (Addeo et al., 1984) and cheese yield (Ariota et al., 2009).

Recently, the effects of CN composite genotypes on BMCP and those of some nongenetic factors on detailed protein composition have been described (Bonfatti et al., 2012a,b). In addition, the protein composition of Mediterranean buffalo milk, namely the percentages of α_{S1}-CN, α_{S2}-CN, and κ-CN in total casein and the percentage ratio of glycosylated-κ-CN to κ-CN, has been reported to be influenced by CN genotypes (Bonfatti et al., 2012c). Associations of specific genotypes at the milk protein genes with the variation in the relative content of the major protein fractions have been indicated to be responsible for the effects of those genotypes on the renneting properties of cow and goat milk (Fox and McSweeney, 2003). As for other dairy species, protein composition may play a role in variation of technological properties of buffalo milk. The aim of this study was to investigate the effects of milk protein composition on variation in pH and BMCP in Mediterranean water buffalo.

MATERIALS AND METHODS

Milk Sampling

Composite milk samples of 2 consecutive evening and morning milkings were collected from October 2010 to April 2011 for 621 Mediterranean water buffaloes reared in 15 farms (Campania region, southern Italy). Only 1 sample was collected for each animal and milk sampling occurred once per herd. Each individual sample was partitioned into 2 subsamples that were frozen immediately after collection and stored at −20°C until transfer to the milk laboratory. Samples were stored in dry ice during the transfer and, at arrival, 1 subsample

Received October 31, 2012.
Accepted March 3, 2013.
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per animal was thawed at 35°C for BMCP analysis, whereas the other was preserved at −40°C until HPLC analysis for the assessment of milk protein composition.

Milk Coagulation Properties

Although milk freezing is undesirable because it may originate significant modification of the CN micelle structure and composition (i.e., separation of β-CN and other CN fractions and release of colloidal calcium phosphate), with unfavorable effects on milk coagulation properties, freezing was necessary to preserve the samples during the transfer from the farms to the laboratory (which took approximately 48 h) and to avoid proteolysis and its effects on the quantification of milk protein fractions.

To ensure the reequilibrium of CN micelles, after thawing, milk samples were kept at room temperature for 2 h before performing the measurement of BMCP. Milk coagulation properties were measured by a computerized renneting meter (CRM-48; Polo Trade, Monselice, Italy), which is based on the swing of a pendulum driven by an electromagnetic field. Variations in the electromagnetic field are recorded during the milk coagulation process. After rennet addition, coagulation takes place and the swing of the pendulum becomes smaller because of the enhanced curd firmness. Details on the equipment used to assess milk coagulation properties have been reported by Dal Zotto et al. (2008), including repeatability and reproducibility of measures.

Samples (10 mL) were preheated at 35°C and 200 μL of rennet (Hansen standard 160, 80% chymosin, 1:14,900; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.6% (vol/vol) in distilled water was added to milk. One measure of rennet-coagulation time (**RCT**), curd-firming time ($\mathbf{K_{20}}$), and curd firmness at 30 min after rennet addition ($\mathbf{A_{30}}$) was obtained per each sample. Measurement of milk pH (pH-Burette 24; Crison Instruments SA, Barcelona, Spain) was carried out immediately before BMCP analysis.

Milk Protein Composition and Genotyping for CN Genes

Contents of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, glycosylated κ-CN (**glyco-κ-CN**), and unglycosylated κ-CN (**glyco-free-κ-CN**), α-LA, and β-LG were measured by reversed-phase HPLC. A detailed description of the procedures for HPLC analysis of buffalo milk protein composition can be found in Bonfatti et al. (2008) and Bonfatti et al. (2013). Total CN content (**TCN**; g/L) was computed as the sum of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, and total κ-CN (i.e., the sum of glyco-κ-CN and glyco-free-κ-CN). Total whey protein content (**WH**; g/L) was calculated as the sum of α-LA and β-LG content. As the frequency distribution of γ-CN was skewed, a logarithmic transformation of γ-CN was computed and used in the statistical analysis. Assessment of genotypes of buffaloes at *CSN1S1* and *CSN3* was also obtained by HPLC (Bonfatti et al., 2013).

Statistical Analysis

Effects of milk protein composition and CN genotypes on variation of pH and BMCP were estimated using different linear models (see Appendix Table A1 for a description of models). These models aimed to investigate initially the effects of groups of protein fractions (casein and whey protein), then those of protein fractions (α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, κ-CN, glyco-κ-CN, glyco-free-κ-CN, α-LA, and β-LG), and lastly to assess whether the effects of protein composition were to be ascribed to variation in enzyme efficiency related to milk acidity.

Preliminary analyses evidenced nonlinear effects of some protein fraction contents on BMCP. Hence, the milk content of each protein or group of proteins was classified as follows: class PR− (content $<\overline{x}$ − SD), class PR−− ($\overline{x}$ − SD ≤ content < $\overline{x}$ − 0.5SD), class PR0 ($\overline{x}$ − 0.5SD ≤ content < $\overline{x}$ + 0.5SD), class PR+ ($\overline{x}$ + 0.5SD ≤ content < $\overline{x}$ + SD), and class PR++ (content ≥$\overline{x}$ + SD).

Additive polygenic effects of the animals or, as an alternative, sire effects were not included in any of the models because reliable pedigree information was not available for all the animals. Indeed, AI is not a common practice in the Italian buffalo population (Rosati and Van Vleck, 2002) and parentage misidentification often occurs (Parlato and Van Vleck, 2012).

Parameters of interest were differences between genotype effects and differences between protein composition class effects, which were computed from model solutions. The marginal posterior densities of parameters of interest, estimated using Bayesian procedures and Gibbs sampling (Sorensen and Gianola, 2002), were used to perform statistical inference. The mean of the marginal posterior density was used as a point estimate of the parameter. From the marginal density, we computed the lower and upper bounds of the 95% highest posterior density interval and the probability of a parameter of being greater (for positive estimates) or lower (for negative estimates) than 0. Differences between genotype or protein composition class effects were considered to be statistically relevant when such probability was greater than 95%.

All Bayesian analyses were performed using bounded uniform distributions as prior densities for the effects included in the models to indicate vague prior knowl-