Bovine Milk Protein Genetic Polymorphisms And Total Milk Protease Activity

S. Chessa[*], O. Bulgari[†], D. Rignanese[†], G. Ceriotti[‡],C. Gigliotti[†], and A.M. Caroli[†*‡]

Introduction

Milk protein system exerts a crucial role from the point of view of both milk nutritional quality and technological properties. It is a dynamic system: milk proteins synthesized by the mammary cells undergo the action of proteolitic enzymes with important effects on milk quality. Proteolysis may have beneficial effects and may be essential for desirable qualities in food products, such as flavor development and texture changes during the ripening of cheese. However, uncontrolled or unwanted proteolysis can adversely affect the quality of foods (Nielsen, 2002), and reduce their shelf-life. The 6 bovine main milk proteins are α_{s1}-, α_{s2}-, β- and κ-CN, β-lactoglobulin (β-LG) and α-lactalbumin (α-LA); they are characterized by an intensive genetic polymorphism, usually affecting α_{s1}-CN, β-CN, κ-CN, and β-LG, and often exerting significant effects on milk composition and technological properties (Caroli *et al.*, 2009). Constituting more than the 95% of the proteins contained in milk, they are the primary target of milk proteolysis, that can be caused by milk native proteases and proteases produced by psychrotrophic bacteria (Kitchen, 1985; Fairban and Law, 1986; Miranda and Gripon, 1986). The main milk native proteases is plasmin; with specific activity on α_{s1}-, α_{s2}- and β-casein (CN) (Grufferty and Fox, 1988; Cassens et al., 1996; Cassens et al., 1999). The proteases produced by psychrotrophic bacteria mainly act on κ-CN, followed by β-CN and, at a lower extent, α_s-CN (Fairban and Law, 1986; Cox, 1993). Generally, high levels of psychrotrophic bacteria in raw milk are required to cause breakdown of protein and fat after pasteurization (Barbano *et al.*, 2006). Relations between the quantitative repartition of milk proteins, their genetic variants and milk proteolitic activity have been scarcely investigated. The aim of this work was to perform a preliminary investigation on the relationships between bovine milk total proteases, the quantitative repartition of the main milk protein fractions and some of the main genetic variants. The overall purpose of this research was to identify novel markers for evaluating milk technological properties by using simple and inexpensive analytical tests.

Material and methods

A total of 180 Italian Friesian herd milk samples were collected from farm devoted to pasteurized milk production, both for conventional and high quality milk. Milk samples were

[*] Istituto di Biologia e Biotecnologia Agraria, CNR, Via E. Bassini 15, 20133 Milano, Italy
[†] Dipartimento di Scienze Biologiche e Biotecnologie, Università degli Studi di Brescia, Viale Europa 11, 25123 Brescia, Italy
[‡] Dipartimento VSA, Università degli Studi di Milano, Via Trentacoste 2, 20134 Milano, Italy

analysed for the proteases activity. A colorimetric method was used to quantify total proteases (Bendicho et al., 2002). The method, based on azocasein substrate, was optimized and standardized for fitting laboratory conditions (Bulgari et al., 2009). It is a cheap and easily applicable method and can be used for routine milk tests. Moreover, IEF was performed (Erhardt et al., 1998) and the gels were acquired and quantified by G:Box (Syngene, model rating, Frederick, MD, USA). Correlation analyses and generalized linear models were fitted to the data by the SAS software (SAS 9.1, SAS Institute, 1999).

Results and discussion

Figure 1 shows a gel obtained by IEF, which allows the simultaneous detection of different milk protein fractions and genetic variants. This technique is usually used to test individual milk samples for genotyping the most common bovine milk protein genetic variants (Caroli *et al*., 2009). Its application to herd milk samples is also interesting because it provides for a fast and cheap genetic overview of the herd as to milk protein variation. First, the attention can be drawn on the occurrence of β-CN A^3 at a rather high frequency in some herds. In fact, it could be easily detectable on the IEF gel, even if bulk milk samples were analysed. This means that β-CN A^3 has an elevated frequency in particular Italian Friesian herds. In all cases, attention must be given to this allele which is often not considered in routine tests depending on the genotyping system used (Bonfatti et al., 2008). Four bands were visible for α_{s1}-CN, 3 of which belonged to α_{s1}-CN B (in pH ascending gradient: 1 = B_1, 2 = B_2 4 = B_3), and only 1 to α_{s1}-CN C (3 = C_2) due to the low frequency of this variant in Holstein Friesian.

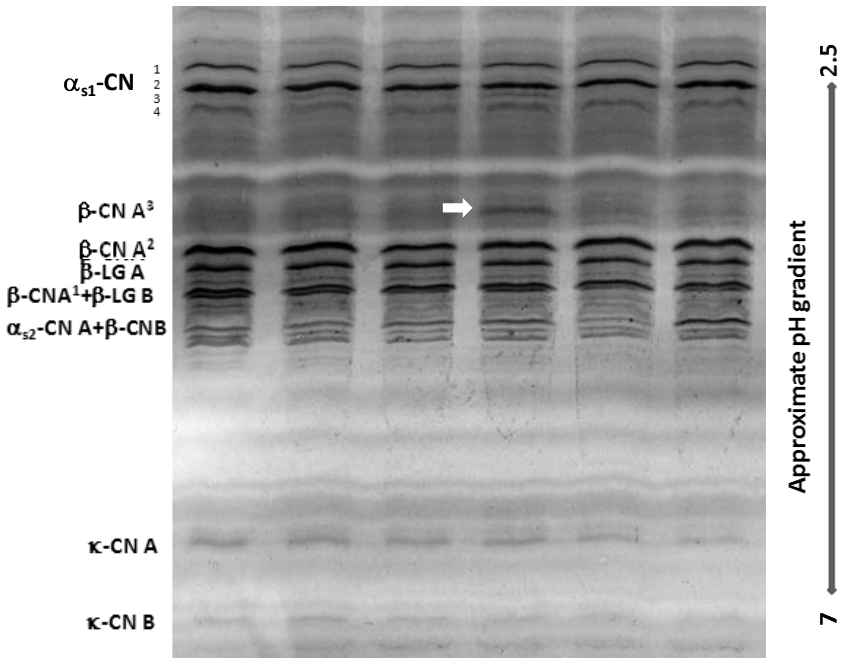

Figure 1: Isoelectrofocusing (IEF) of 6 herd milk samples. White arrow: β-CN A^3.

For κ-CN, it was possible to calculate the ratio between κ-CN A % and B % and the total A + B %, in order to have a hint about the gene frequencies within each herd. The descriptive statistics of the analysed variables are shown in Table 1. It is to point out that κ-CN A includes also κ-CN E, since the very close IEF migration of the 2 variants makes difficult their distinction in bulk milk typing. In the central part of the gel, the close migration of different fractions and variants allows the quantification of groups of them only. Some relations were identified between the total proteases activity and the quantitative repartition of the main milk protein fractions and genetic variants (Table 1). Significant negative correlations with proteases activity were found for β-CN A^2, β-LG A and β-CN A^1+β-LG B %, whereas significant positive relationships occurred with γ-CN (C-terminal portions of β-CN released by milk proteolysis) and κ-CN %. These effects can be related to the higher activity of native proteases in the analysed milk, given that proteases from psychrotrophic bacteria mainly act on κ-CN. Moreover, interesting results were found when considering the differences of milk protein repartition as a function of milk destination. In particular, κ-CN % was statistically higher in high quality than conventional milk (difference: +1.66 %, $P < 0.01$). This effect was more evident considering κ-CN A% only ($P < 0.001$). A recent method was developed by Summer et al. (2010) for quantifying the κ-CN B content in bovine milk. The authors suggested the introduction of κ-CN B content into quality payment systems for cheese milk. At the light of our results, it should be considered the developing of specific tests evaluating the overall content of κ-CN fraction. The chromatography method developed by Bonfatti et al. (2008) is an example of a valid milk protein quantification tool, even if more routinary tests should be developed for practical use (De Marchi et al., 2009).

Table 1: Descriptive statistics of the analysed variables and Pearson correlation coefficient (R) of each variable with total proteases.

Variable	Mean	SD	R
Total proteases mU/mL	9.52	2.36	1.00
α_{s1}-CN B_1 (%)	9.72	2.04	0.01
α_{s1}-CN B_2 (%)	20.77	3.56	0.09
α_{s1}-CN C_2 (%)	2.16	2.54	-0.05
α_{s1}-CN B_3 (%)	5.64	2.47	0.11
β-CN A^3 (%)	1.08	3.56	-0.05
β-CN A^2 (%)	10.04	2.95	-0.25^{**}
β-LG A (%)	9.29	1.97	-0.28^{***}
β-CN A^1+β-LG B (%)	12.80	3.43	-0.23^{**}
α_{s2}-CN A+β-CN B (%)	12.14	3.39	0.03
κ-CN A (%)	10.66	3.83	0.26^{***}
κ-CN B (%)	3.71	1.97	$0.15^{\S}$
α_{s1}-CN (%)	38.29	4.90	0.10
κ-CN B (%)	3.71	1.97	$0.15^{\S}$
γ-CN (%)	3.05	1.15	0.17^{*}
κ-CN (%)	14.38	4.80	0.27^{***}
κ-CN $A^{(ratio)}$	0.74	0.09	-0.00

$^{(ratio)}$ = κ-CN A/(A+B); A = A+E: § P>0.1; * 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001.

Conclusion

Statistical relations were found between total milk proteases activity and milk protein repartition in this preliminary study. Moreover, the simultaneous use of the methods here used could be further investigated for assessing the practical applications of these cheap analytical tools for bulk milk testing. The significant effect found for milk destination on κ-CN % content is a clear hint of the applicability of the IEF analysis for milk composition evaluation, as well as for the estimation of the frequency of particular variants in the different herds. Appropriate modifications of the IEF technique could be realized in order to establish more suitable analytical conditions for studying the proteolitic changes of milk proteins. Milk quality payment system should include further simple and inexpensive tests to improve the definition of milk quality properties, also on the basis of its final destination.

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