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CHARACTERIZATION OF MILK COAGULATION ABILITY IN BULK MILK SAMPLES

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ABSTRACT

Basic requirements for cheese-making production are milk coagulation properties (MCP), which can be measured as: (i) rennet coagulation time (RCT, min), (ii) curd-firming time (k_{20}, min), and (iii) curd firmness ($a_{30,}$ mm). The aim of this study was to investigate sources of variation of coagulation properties in bulk milk from dairy cattle farms of northern Italy. A total of 1,570 samples were collected from 436 herds that delivered milk to 4 dairy cooperatives, and analyzed for quality traits and MCP. About 4% of total samples did not coagulate within 30 min. Rennet coagulation time, k_{20}, and a_{30} averaged 18.83 min, 6.85 min, and 26.97 mm, respectively. Milk coagulation properties were analyzed through a general linear model which included the fixed effects of dairy cooperative, herd nested within dairy cooperative, year and season of sampling, and milk quality traits, inserted as class factors in the model. Dairy cooperative and herd effects were the major sources of variation in explaining the variation of MCP ($P < 0.001$). Season of sampling was statistically significant for RCT and k_{20} ($P < 0.05$), showing the best results in summer, whereas year of sampling was statistically significant only for RCT ($P < 0.001$). Rennet coagulation time was influenced by titratable acidity and bacterial count ($P < 0.05$), and k_{20} and a_{30} by casein content and titratable acidity ($P < 0.01$).

Key words: bulk milk / coagulation properties / dairy industry / cheese making / milk acidity

1 INTRODUCTION

Milk and dairy products are key components for Italian agricultural and food sectors, and in several areas of the country they represent the primary source of income for farmers. At present, 70% of the milk available in Italy is used for cheese production and about 35% is transformed into PDO products (Pieri, 2011). More than 83% of cow milk is obtained in northern Italy, where Veneto Region represents the third producer (Pieri, 2011). In this Region cheese-making is performed by several dairy cooperatives that process the milk collected from dairy farms.

As reported by Summer *et al.* (2002), basic requirements for cheese-making are milk coagulation properties (MCP), which are: (i) rennet coagulation time (RCT, min), (ii) curd-firming time (k_{20}, min), and (iii) curd firmness ($a_{30,}$ mm). These parameters can be determined by Formagraph (Annibaldi *et al.*, 1977; Zannoni and Annibaldi, 1981; MacMahon and Brown, 1982). The milk clotting properties are important both with regard to quality and yield of cheese (Wedholm *et al.*, 2006); in fact, a firmer curd at cutting is positively correlated to cheese yield (Aleandri *et al.*, 1989; Martin *et al.*, 1997).

Milk coagulation properties are affected by different factors such as milk quality, breed, season, and herd. Several studies have investigated the relationship between MCP and milk quality traits both from phenotypic and genetic point of view. Jõudu *et al.* (2008) reported that an increase of milk protein and casein contents reduced RCT. Politis and Ng-Kwai-Hang (1988) and Okigbo *et al.* (1985b) found an improvement of MCP associated to decreasing values of somatic cell count (SCC). Okigbo *et al.* (1985a) and Formaggioni *et al.* (2001) reported that

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MCP were better when associated to decreasing values of pH and increasing values of titratable acidity (TA), respectively. Besides milk quality, breed of cow and herd are important sources of variation for MCP. De Marchi *et al.* (2007) reported relevant differences among five dairy cattle breeds for MCP measured on bulk milk samples. A strong breed effect was also confirmed in individual milk samples (Mariani *et al.*, 1997, 2002; Tyrisevä *et al.*, 2002). Finally, the season affects MCP even if contradictory findings are available in literature. Chládek *et al.* (2011) found that season significantly affected MCP; in particular, these authors reported better RCT in summer than in other seasons, and this trend was confirmed also by Barlowska *et al.* (2012). Opposite results were obtained by Jõudu *et al.* (2008) and De Marchi *et al.* (2007) showing worst MCP for milk samples collected in summer.

The aim of this study was to investigate sources of variation of MCP in bulk milk destined to cheese production.

2 MATERIAL AND METHODS

2.1 DATA COLLECTION AND LABORATORY ANALYSES

A total of 1,570 bulk milk samples were collected between June 2008 and November 2009 from 436 dairy herds (mainly rearing Holstein-Friesian cows) that delivered milk to 4 dairy cooperatives. After collection, samples (100 mL) without preservative were transferred to the Milk Quality Laboratory of Veneto Agricoltura (Thiene, Italy) and assessed for fat, protein, and casein contents (MilkoScan[tm] 6000, Foss Electric A/S, Hillerød, Denmark), SCC (Cell Fossomatic 250, Foss Electric A/S, Hillerød, Denmark), pH, and TA expressed in Soxhlet-Henkel degrees (°SH/50 ml; Crison Compact D, Crison Instruments SA, Alella, Spain) as proposed by Anonymous (Sauregradbestimmung …, 1963).

Analysis of MCP was carried out following Cassandro *et al.* (2008); briefly, milk samples (10 mL) were heated to 35 °C and 200 μL of rennet (Hansen standard 190 with 63% of chymosin and 37% of pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted 1.6% in distilled water, was added to milk. Milk coagulation properties were determined through a computerized renneting meter for 30 min. Measurements recorded were rennet coagulation time (RCT, min), curd-firming time (k_{20}, min), and curd firmness (a_{30}, mm). Rennet coagulation time is the time between the addition of the clotting enzyme to the milk and the beginning of coagulation, k_{20} corresponds to the time required to achieve 20 mm of firmness, and a_{30} expresses the millimeters of curd obtained at 30 min from rennet addition.

2.2 STATISTICAL ANALYSES

Milk samples that did not coagulate within 30 min (3.95% of total records) were discarded from the database prior to statistical analysis. An analysis of variance was performed on MCP using the GLM procedure of SAS (SAS, 2008) according to the following linear model:

$$y_{ijklmnopqr} = \mu + DC_i + H_j(DC_i) + YS_k + SS_l + CAS_m + FAT_n + TA_o + SCC_p + BC_q + \varepsilon_{ijklmnopqr},$$

where $y_{ijklmnopqr}$ is RCT, k_{20}, or a_{30}; μ is the overall mean; DC_i is the fixed effect of the i*th* dairy cooperative (i = 1 to 4); H_j is the fixed effect of the j*th* herd nested within dairy cooperative (j = 1 to 436); YS_k is the fixed effect of the k*th* year of sampling (k = 2008, 2009); SS_l is the fixed effect of the l*th* season of sampling (l = 1 to 4); CAS_m is the fixed effect of the m*th* class of casein content of milk (m = 1 to 5); FAT_n is the fixed effect of the n*th* class of fat content of milk (n = 1 to 5); TA_o is the fixed effect of the o*th* class of titratable acidity of milk (o = 1 to 5); SCC_p is the fixed effect of the p*th* class of somatic cell content of milk (p = 1 to 5); BC_q is the fixed effect of the q*th* class of bacterial count (q = 1 to 5); and $\varepsilon_{ijklmnopqr}$ is the random residual effect ~N(0, $\sigma^2\varepsilon$). The herd effect was tested on the error line of herd within dairy cooperative variance. Significance was set at $P < 0.05$.

3 RESULTS AND DISCUSSION

Rennet coagulation time, k_{20}, and a_{30} averaged 18.83 min, 6.85 min, and 26.97 mm, respectively (data not shown), and the coefficient of variation ranged from 0.20 to 0.30, highlighting a good variation of MCP. De Marchi *et al.* (2007) reported similar values for herd bulk milk samples collected on 5 dairy cattle breeds in northern Italy. The mean values for RCT, k_{20}, and a_{30} were not close to those recommended by Zannoni and Annibaldi (1981) for cheese-making; these authors reported optimal values of 13 min for RCT, 9 min for k_{20}, and 35 mm for a_{30}. Sixty two samples (3.95% of total records) did not coagulate within 30 min from the beginning of the analysis. Non-coagulating milk samples ranged between 7.5 and 13.2% in Ikonen *et al.* (2004), Tyrisevä *et al.* (2004), and Cassandro *et al.* (2008), i.e., they were much higher than findings from our study. A possible explanation of such difference is that we consider bulk milk, whereas previous studies dealt with individual samples. It is likely that

***Table 1:** Results from ANOVA for milk coagulation properties of bulk milk samples*

	Trait[1]					
	RCT, min		k_{20}, min		a_{30}, mm	
Effect	F	P-value	F	P-value	F	P-value
Dairy cooperative[2]	25.03	<0.001	9.36	<0.001	25.57	<0.001
Herd (within dairy cooperative)	1.86	<0.001	1.57	<0.001	1.83	<0.001
Year of sampling	19.08	<0.001	1.12	0.290	0.07	0.797
Season of sampling	13.75	<0.001	2.66	0.047	1.51	0.211
Casein, %	0.71	0.585	4.84	0.001	5.88	<0.001
Fat, %	0.58	0.676	1.06	0.376	1.47	0.209
Titratable acidity, °SH/50 mL	14.31	<0.001	4.78	0.001	13.63	<0.001
Somatic cell count, cells/mL	2.31	0.056	1.13	0.339	0.97	0.422
Bacterial count, cells/mL	2.48	0.042	1.70	0.148	1.56	0.183
R^2	0.52		0.52		0.52	
RMSE[3]	3.03		1.65		6.72	

[1] RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness; [2] Tested on herd within dairy cooperative variance;
[3] RMSE = root mean square error.

the variation in bulk milk is reduced as a consequence of the blending of milk from different cows.

Results from the analysis of variance for MCP are shown in Table 1. The coefficient of determination (R^2) was the same for all the studied traits (0.52). The dairy cooperative and herd effects were highly significant ($P < 0.001$) in explaining the variation of MCP. Year of sampling was statistically significant ($P < 0.001$) only for RCT, whereas season of sampling was significant for RCT and k_{20} ($P < 0.05$). The importance of herd effect on MCP was reported by several authors (Ikonen *et al.*, 2004; Tyrisevä *et al.*, 2004; De Marchi *et al.*, 2007; Vallas *et al.*, 2010; Barlowska *et al.*, 2012). Regarding the effect of season of sampling on MCP, Barlowska *et al.* (2012), Chládek *et al.* (2011), and Hanuš *et al.* (2010) reported the shortest RCT in summer, whereas De Marchi *et al.* (2007) found shorter RCT and higher a_{30} in fall.

The effect of milk composition on MCP is reported in Table 1. Titratable acidity and bacterial count significantly ($P < 0.05$) influenced RCT, whereas SCC approached significance ($P < 0.10$) for this trait. Casein content and TA significantly influenced ($P < 0.01$) k_{20} and a_{30}. Rennet coagulation time decreased with increasing values of TA, and increased with increasing values of SCC (Fig. 1). Better values of k_{20} were associated to higher content of casein in milk and to higher TA (Fig. 2). Finally, a_{30} in-

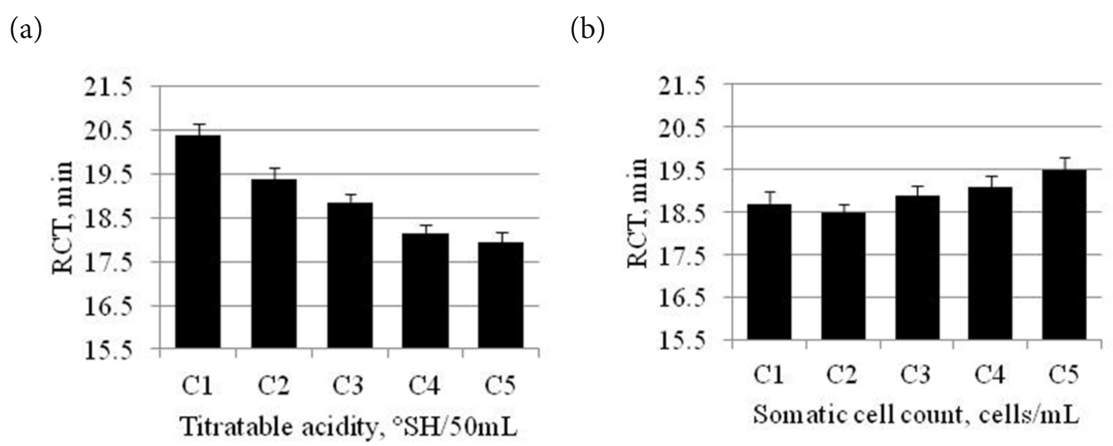

***Figure 1:** Least squares means of rennet coagulation time (RCT, min) across classes of (a) titratable acidity and (b) somatic cell count. Titratable acidity (°SH/50 mL): C1 < 3.00; C2 3.00 to 3.20; C3 3.20 to 3.40; C4 3.40 to 3.60; C5 > 3.60. Somatic cell count: C1 < 100,000; C2 100,000 to 200,000; C3 200,000 to 300,000; C4 300,000 to 400,000; C5 > 400,000.*

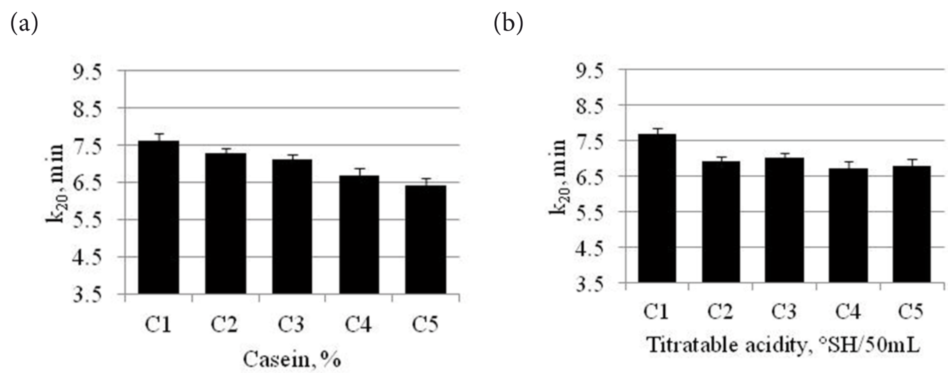

Figure 2: *Least squares means of curd-firming time (k20, min) across classes of (a) casein content and (b) titratable acidity. Casein content: C1 < 2.37; C2 2.37 to 2.52; C3 2.52 to 2.67; C4 2.67 to 2.82; C5 > 2.82. Titratable acidity (°SH/50 mL): C1 < 3.00; C2 3.00 to 3.20; C3 3.20 to 3.40; C4 3.40 to 3.60; C5 > 3.60.*

creased with increasing values of casein content and TA (Fig. 3). These results were previously confirmed by De Marchi *et al*. (2007) who found lower RCT and k_{20} values associated to higher TA of bulk milk. The fundamental role of TA in explaining the variation of MCP was also reported by Formaggioni *et al*. (2001) in bulk milk samples and Okigbo *et al*. (1985a) in individual milks.

The relationship between MCP and composition and acidity of milk has also been studied at cow level. Favorable association of technological properties with acidity was found by Cassandro *et al*. (2008) who reported phenotypic and genetic correlations of −0.43 and −0.50 between RCT and TA, and 0.41 and 0.66 between a_{30} and TA. The same authors estimated phenotypic and genetic correlations of 0.17 and 0.25 between RCT and SCC, and −0.14 and −0.40 between a_{30} and SCC, in agreement with estimates from a previous study by Ikonen *et al*. (2004). Similar findings were also reported by Politis and Ng-Kwai-Hang (1988) who detected a worsening of MCP when values of SCC exceeded 500,000 cells/mL. The relationship between casein content and MCP has been investigated by several authors who reported a positive association between these traits: an increase of milk casein content is associated to an increase of the strength of the coagulum (Mariani *et al*., 1976; Okigbo *et al*., 1985a; Politis and Ng-Kwai-Hang, 1988; Ikonen *et al*., 1997; Tyrisevä *et al*., 2003, 2004).

4 CONCLUSIONS

This study showed the strong influence of dairy cooperative and herd effects on MCP measured on bulk milk. The best MCP were obtained in summer; however, the season effect should be further investigated as contradictory results have been found in literature. Quality of milk affected MCP. In particular, TA strongly affected RCT, k_{20}, and a_{30}, and casein content had a large influence on k_{20} and a_{30}.

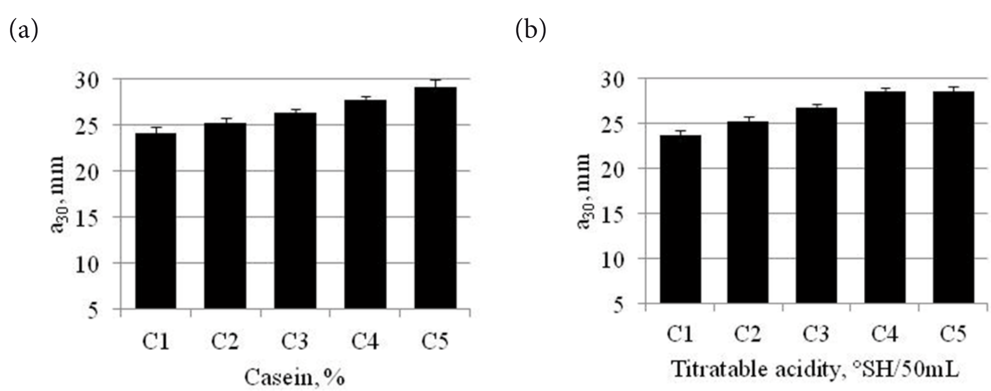

Figure 3: *Least squares means of curd firmness (a30, mm) across classes of (a) casein content and (b) titratable acidity. Casein content: C1 < 2.37; C2 2.37 to 2.52; C3 2.52 to 2.67; C4 2.67 to 2.82; C5 > 2.82. Titratable acidity (°SH/50 mL): C1 < 3.00; C2 3.00 to 3.20; C3 3.20 to 3.40; C4 3.40 to 3.60; C5 > 3.60.*

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