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VARIATIONS OF NITROGEN FRACTIONS, PROTEOLYSIS AND RENNET-COAGULATION PROPERTIES OF MILK WITH DIFFERENT SOMATIC CELL VALUES[1]

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Parole chiave:
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RIASSUNTO

Sono state studiate le variazioni di alcune componenti azotate e delle principali caratteristiche di coagulazione presamica del latte in rapporto al contenuto di cellule somatiche. La ricerca è stata condotta su 60 campioni di latte individuale classificati in base al loro contenuto di cellule somatiche: basso (L), fino a 250000; medio (M), da 250000 a 750000; elevato (H), sopra 750000 unità/mL. Il latte ad elevato contenuto di cellule somatiche è risultato povero di lattosio (4,85 L vs 4,66 M vs 4,57 H, g per 100 g; P<0,0001), ricco di cloruri (98,5 L vs 111,5 M vs 117,9 H, mg Cl^- per 100 g; P<0,0001) e contraddistinto da un basso indice di caseina (77,36 L vs 76,16 M vs 74,87 H; P<0,001). I latti individuali ad elevato contenuto di cellule somatiche hanno fatto registrare una maggiore proteolisi con relativo accumulo di proteoso-peptoni (N proteoso-peptoni: 11,5 L vs 15,9 M vs 17,6 H, mg per 100 g; P<0,05). Gli stessi, inoltre, contraddistinti da una minore acidità titolabile (3,61 L vs 3,38 M vs 3,17 H; °SH/50mL; P<0,0001) e da un pH più elevato (6,64 L vs 6,69 M vs 6,73 H; unità; P<0,01), sono risultati caratterizzati da un tempo di rassodamento significativamente più lungo (3,90 L vs 4,64 M vs 7,74 H; min; P<0,001), chiaro indice di una minore capacità di aggregazione delle micelle di paracaseina, conseguente ad una profonda alterazione del sistema micellare.

ABSTRACT

The variations of some nitrogen components and the principal rennet-coagulation properties of milk in relation to somatic cell content were studied. The research

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was carried out on 60 individual milk samples grouped according to their somatic cell count: low (L), up to 250000; moderate (M), from 250000 to 750000; high (H), over 750000 units/mL. Milk with a high somatic cell count was poor in lactose (4.85 L vs 4.66 M vs 4.57 H, g per 100 g; $P<0.0001$), rich in chloride (98.5 L vs 111.5 M vs 117.9 H, mg Cl^- per 100 g; $P<0.0001$) and characterised by a low casein number (77.36 L vs 76.16 M vs 74.87 H; $P<0.001$). Individual milk samples with a high somatic cell content showed a higher degree of proteolysis, as demonstrated by the relative greater accumulation of proteose-peptones (proteose-peptone N: 11.5 L vs 15.9 M vs 17.6 H, mg per 100 g; $P<0.05$). Moreover, the same samples were characterised by a lower titratable acidity (3.61 L vs 3.38 M vs 3.17 H; °SH/50mL; $P<0.0001$), a higher pH values (6.64 L vs 6.69 M vs 6.73 H; units; $P<0,01$) and showed a significantly longer curd firming time (3.90 L vs 4.64 M vs 7.74 H; min; $P<0.001$), clear index of a lower aggregation capacity of the para-casein micelles, consequence of an alteration of the micellar system.

INTRODUCTION

Quantitative and qualitative variations of milk casein markedly affect the rheological properties of curd, with repercussion on texture and, consequently, on cheese quality.

The rennet-coagulation aptitude of milk plays a key role in Parmigiano-Reggiano cheese manufacture, whose production is based on the formation and syneresis of a lactic-rennet curd [1].

In this regard, the structural characteristics of the micellar system are of primary interest. These characteristics are mainly of genetic origin. The integrity of the native casein, which is mainly related to the functional condition of the mammary gland, plays a relevant role as well [2].

In particular physiological conditions (e.g. end of lactation) and, above all, as a consequence of mammary infections, functional alterations are mainly related to hydrolytic degradation of casein by the alkaline protease of milk, with strong repercussion on the processing properties of milk [3-8]. Among degradation products, γ-caseins and proteose-peptones which arise from the selective hydrolysis of β-casein [9, 10] have to be considered.

The aim of this research was to study the variations of nitrogen fractions (those related to the integrity state of the micellar system) and rennet-coagulation characteristics of individual milk samples in relation to the content of somatic cells.

MATERIALS AND METHODS

The research was carried out on 60 “individual” milk samples. The samples were grouped in 3 classes according to their somatic cell count (SCC): low (L), up to 250 000 cell/mL, moderate (M), from 250000 to 750000 cell/mL, high (H), above 750000 cell/mL.

Milk samples were collected throughout a one year period, from Italian Friesian cows reared in both small and medium size herds which were located in the province of Parma, in both the plain and in hill zones.

On each milk sample the following parameters were determined:

- pH with a potentiometer, titratable acidity with 0.25 M-NaOH using the Soxhlet-Henkel method [11]

- Fat and lactose by infrared analysis [12] with a Milk-o-Scan 134 A/B (Foss Electric, DK-3400 Hillerød, Denmark)

- Somatic cell count (SCC), by means fluoro-opto-electronic method [13] with Fossomatic 250 (Foss Electric, Hillerød, Denmark);

- Total nitrogen (TN) in milk and non-casein nitrogen (NCN) in acid whey at pH 4.6 were determined by the Kjeldahl method [14]; from which casein nitrogen (CN = TN - NCN) and casein number (CN*100/TN)

- Proteose-peptone nitrogen (PPN) according to Aschaffenburg and Drewry [14]. Briefly, anhydrous sodium sulphate was added to pH 4.6 acid whey of milk to precipitate proteose-peptone fraction. Its content was determined as difference.

- Milk coagulation properties: to milk samples (10 mL) 0.2 mL (1 : 100) a rennet solution (1 : 19 000; Chr. Hansen, I-20094 Corsico MI, Italy) was added. Milk clotting time (r), curd firming time (k_{20}) and curd firmness (a_{30}), were measured at 35 °C [15] using a Formagraph (Foss Electric): r is the time from the addition of rennet to the onset of gelation; k_{20} is the time from the onset of gelation till the signal attains a width of 20 mm; a_{30} is the width of the signal 30 min (a_{30}) after the addition of rennet.

Statistical significance of differences were tested with univariate ANOVA considering as fixed factors somatic cell class (low, moderate and high).

RESULTS AND DISCUSSION

The contents of lactose and chloride were different ($P<0.05$) among L, M and H individual milk samples. Lactose was lower in M and H milks than in L milk (4.85 L vs 4.66 M vs 4.57 H, g/100 g; $P<0.0001$). On the other hand, M and H milks were characterised by a higher chloride content than L milk (98.5 L vs 111.5 M vs 117.9 H, mg Cl^- /100 g; $P<0.0001$). In H milk lactose content was 6% lower and the chloride content resulted 20% higher than in L milk.

As far as nitrogen fractions are concerned, the increase of somatic cell count was related to a progressive increase of non-casein nitrogen content (112.8 L vs 120.0 M vs 125.5 H, mg/100 g; $P<0.01$). A positive correlation between somatic cell values and pH 4.6 soluble nitrogen was reported by Somers et al. [16] in a study focused on individual milk samples.

On the other hand, variations of casein nitrogen content were not significant, even if milk with the highest value of somatic cells showed, on average, a lower concentration of casein (374.3 mg/100 g).

The combined effects of these two phenomena determined significant variations of the casein number among L, M and H milks (77.36 L vs 76.16 M vs 74.87 H; $P<0.001$). In fact, in L milk the value of casein number was markedly lower when compared to H milk.

When compared to L milk, M and H milks were characterised by a higher content of proteose-peptone N (11.5 L vs 15.9 M vs 17.6 H, mg/100 g; P<0.05).

Proteose-peptone represented an increasing quota of casein nitrogen as milk somatic cell content increased. This suggests a possible degradation of the organic component of the micellar system by the plasmin.

This latter hypothesis is indirectly supported by the observations reported by Urech et al. [17]. These authors reported an increase of the relative proportion of γ-casein in quarter milk samples with high somatic cell count with respect to quarter milk samples CMT (California Mastitis Test) negatives and with low somatic cell count.

The titratable acidity values were markedly different (P<0.0001) among L, M and H individual milks (3.61 L vs 3.38 M vs 3.17 H; °SH/50mL) (Table1).

The values of pH resulted higher in H milk than L and M milks (6.64 L vs 6.69 M vs 6.73 H; units; P<0.01).

In general, M milk and, in particular, H milk showed physico-chemical alterations, particularly at the level of acidity, such as to influence the rennet-coagulation properties. In fact, both milks were characterised by higher clotting time (15.45 L vs 18.38 M vs 20.43 H; min; P<0.01) when compared to L milk.

Differences in curd firming time are remarkable (3.90 L vs 4.64 M vs 7.74 H; min; P<0.001): H milk value was almost twice with respect to L milk value. This is an index of deep modifications at the level of the aggregation capacity of paracasein micelles of H milk, probably related to structural alterations of the micellar system of this milk.

These observations agree with those reported by Srinivasan and Lucey [18] about the marked alterations of rheological properties of rennet-curd as a consequence of the hydrolytic action of the plasmin on native micellar casein.

The variations of curd firmness measured 30 minutes after rennet addition were remarkable as well (34.6 L vs 28.6 M vs 23.3 H; mm; P<0.01).

CONCLUSIONS

A marked alteration of those milk characteristics related to functional conditions of the mammary gland was observed in milks with moderate and high somatic cell content.

As far as nitrogen fractions, an increase of non-casein nitrogen and, as a consequence, a decrease of casein number was observed. A significant increase of the proteose-peptone content in the milk and its proportion, with respect to casein, were registered as well. This latter variation clearly demonstrate that the increase of the proteolytic activity in mastitic milk is the result of the increased activity of the alkaline protease.

The worsening of rennet-coagulation properties of milk with a high content of somatic cell is probably the result of an alteration of the integrity of the micellar system.

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rheological properties of rennet-induced skim milk gels. J. Dairy Sci., 85, 1070-1078.

Table 1 – Individual milk samples: basic composition, nitrogen fraction distribution, pH, titratable acidity and rennet-coagulation parameters according to three somatic cell levels. Mean±SD.

Tabella 1 *- Latti individuali: caratteristiche di base, ripartizione delle frazioni azotate, pH, acidità titolabile e parametri di coagulazione secondo tre differenti livelli di cellule somatiche. Media±DS*

		Low *Basso* (n = 15)	Moderate *Medio* (n = 15)	High *Elevato* (n = 15)	P
Somatic cells SCC *Cellule somatiche SCC*	10^3/mL	110± 52	529± 79	1699± 876	—
Protein TN x 6.38 *Proteina TN x 6,38*	g/100g	3.18± 0.20	3.21± 0.19	3.19± 0.20	NS
Fat *Grasso*	g/100g	3.03± 0.97	3.30± 0.60	3.39± 0.67	NS
Lactose *Lattosio*	g/100g	4.85± 0.11 b	4.66± 0.15 a	4.57± 0.16 a	****
Chloride, Cl^- *Cloruri, Cl^-*	mg/100g	98.5± 8.6 a	111.5± 8.8 b	117.9± 9.4 b	****
Total N TN *N totale TN*	mg/100g	498.3± 31.3	503.0± 30.1	499.8± 30.8	NS
Casein N CN *N caseina CN*	mg/100g	385.5± 26.0	383.0± 22.3	374.3± 25.6	NS
Non-casein N NCN *N non caseinico NCN*	mg/100g	112.8± 11.3 a	120.0± 8.7 ab	125.5± 10.9 b	**
Non-protein N NPN *N non proteico NPN*	mg/100g	25.3± 3.8	24.7± 3.2	24.0± 2.7	NS
Proteose-peptone N PPN *N prot-peptoni PPN*	mg/100g	11.5± 4.9 a	15.9± 5.0 ab	17.6± 8.0 b	*
CN / TN	%	77.36± 1.83 c	76.16± 0.68 b	74.87± 1.72 a	***
PPN / CN	%	3.01± 1.32 a	4.16± 1.31 ab	4.73± 2.30 b	*
pH		6.64± 0.08 a	6.69± 0.06 a	6.73± 0.06 b	**
Titratable acidity *Acidità titolabile*	°Soxhlet-Henkel/50 mL	3.61± 0.24 c	3.38± 0.25 b	3.17± 0.19 a	****
Clotting time r *Tempo coagulazione r*	min	15.45± 3.10 a	18.38± 4.38 b	20.43± 3.69 b	**
Curd firming time k_{20} *Tempo rassodamento k_{20}*	min	3.90± 1.20 a	4.64± 1.65 a	7.74± 4.20 b	***
Curd firmness a_{30} *Consistenza coagulo a_{30}*	mm	34.56± 5.38 b	28.60± 8.57 ab	23.33± 9.85 a	**

NS, P>0.05; * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001

a, b, c: values in the same row with different letters are different for P<0.05

a, b, c: valori con lettere diverse nella stessa riga differiscono per P<0,05.