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Effect of milk urea content on characteristics of matured Reblochon cheeses

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Summary — According to a crossed experimental design, two groups of 14 Montbéliarde dairy cows received in the winter two hay-based diets, with a normal or a high nitrogen level, obtained by substituting in the diet 2.5 kg of soybean meal to 2.5 kg of cereal concentrate. During the last week of the two experimental periods of 3 weeks, four batches of Reblochon cheese were manufactured with three different milks: control milk, milk from soya-fed cows and control milk with 0.25 g/L of added urea. Twenty-four batches were manufactured under identical conditions with full fat raw milk. A high nitrogen level diet increased milk urea content, which rose from 0.24 to 0.49 g/L, and decreased the protein content slightly. Rennet clotting time and curd firming time were slightly higher in the high nitrogen level. Milk fat, casein, total and soluble Ca and P were not modified by the diet. A high milk urea content, whether it comes from addition to milk or from feeding, leads to a lower acidification rate in mould and at demoulding to moister ripened cheeses. Nitrogen fractions, pH, and Ca content of cheeses were not modified by milk urea content. While eating, compared with control cheeses, cheeses from soya-fed cows were significantly less firm, less pasty, less chalky. Their taste was creamier, less acid and sweeter. Cheeses from added urea milk were close to cheeses from soya-fed cows in their chemical composition and texture, and to control cheeses in their taste. This suggests that milk urea, whether it comes from addition to milk or from feeding, influences directly acidification kinetics, chemical composition and texture characteristics of matured cheeses, however, urea is not directly involved in taste differences.

diet nitrogen level / milk urea / cheese / Reblochon / sensory characteristic

Résumé — Effet de la teneur du lait en urée sur les caractéristiques des reblochons affinés. Deux lots de 14 vaches laitières de type Montbéliardes ont reçu au cours de l'hiver, selon un dispositif expérimental croisé, deux régimes à base de foin avec un niveau d'apports azotés normal (C) ou élevé (S) obtenu en substituant 2,5 kg de tourteau de soja à 2,5 kg de céréales dans la ration. A huit reprises, des reblochons ont été réalisés dans des conditions technologiques contrôlées et identiques

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d'une fabrication à l'autre, à partir de trois laits différents : lait des animaux recevant le régime C, lait des animaux recevant le régime S et lait C dans lequel 0,25 g/L d'urée a été ajouté (lait AU). Le régime S a provoqué une augmentation de la teneur du lait en urée, qui est passée de 0,24 g/L (régime C) à 0,49 g/L et une légère détérioration du taux protéique et de l'aptitude à la coagulation. Comparativement aux fromages C, les fromages S ont été significativement moins fermes, moins pâteux, moins plâtreux, plus crémeux, moins acides et plus doux. Les fromages AU se rapprochent des fromages S en ce qui concerne leur composition chimique et leur texture et des fromages C en ce qui concerne leur goût. Quelle que soit l'origine de l'urée dans le lait, les laits les plus riches en urée présentent une acidification plus lente au cours des premières heures suivant le moulage, qui peut expliquer en partie les différences de composition chimique et de texture observées sur les fromages affinés.

alimentation azotée / urée du lait / fromage / reblochon / caractéristique sensorielle

INTRODUCTION

The chemical and sensory properties of matured cheese depend on many factors linked, on the one hand, to the technology employed and, on the other hand, to the original characteristics of the milk. Among the numerous characteristics of milk that may influence matured cheese properties, urea content, which depends mainly on nitrogen balance of the diet (Journet et al, 1975; Oltner et al, 1985; Vérité et al, 1995) seems to play an important role (Gravert et al, 1990; Martin and Coulon, 1995). An increase in milk urea content leads to partial dissociation of all casein components, calcium and phosphate, into the soluble phase (Muir and Sweetsur, 1977; Ali et al, 1980; Dalgleish et al, 1987) and decreased milk clotting ability (Mariani et al, 1992). Some authors show that urea inhibits activity of starter culture (Podhorsky and Cvak, 1989) and modifies fresh cheese's chemical composition (Guinot-Thomas, 1992). Nevertheless, there is little information on the influence of milk urea content on ripened cheese characteristics, the information available coming only from observations in farms (Martin and Coulon, 1995) or from adding urea to milk at a high and non-physiological level (1 g/L) (Millet, 1989).

The aim of this study was to test the influence of urea content of milk on its suitability for cheesemaking. We compared the

chemical composition and sensory properties of matured Reblochon cheeses manufactured in a pilot plant using milk with a normal urea content (0.25 g/L) and milk with a high urea content (0.49 g/L) obtained on the one hand by cows fed a high nitrogen supply and on the other hand by adding urea to the normal milk.

MATERIALS AND METHODS

Experimental design

Twenty-eight Montbéliardes cows from a farm in the Haute-Savoie area (France) were used in this experiment. Two groups of cows (14 each) were formed on the basis of production performance during a pre-experimental period of 2 weeks and on the genetic variants of κ -casein (respectively 25 and 29% of κ -casein B allelic frequency in the two groups). During the entire experiment, cows were fed a hay-based diet ad libitum consisting of native mountain grassland and 3 kg of dehydrated lucerne, supplemented with a mixture of rolled cereals and soybean meal fed twice daily (table I). The quantities of concentrate were determined for the whole experiment, so as to cover the maintenance and milk production requirements monitored over the pre-experimental period, according to Inra recommendations (Jarrige, 1989). The experiment was conducted according to a crossed experimental design over two experimental periods (3 weeks each) during which each group of cows was successively fed a diet with a control (C) or high (soya, S) nitrogen level. Animals of group C

Table 1. Composition and nutritive value of feeds.
Composition et valeur nutritive des aliments.

	Hay	Dehydrated lucerne	Cereal concentrate ¹	Soybean meal
Dry matter (DM) (g/kg)	87.8	90.0	87.0	88.0
Crude protein (g/kg DM)	112	230	117	430
Crude fiber (g/kg DM)	369	180	61	100
UFL (/kg DM) ²	0.62	0.95	1.02	0.94
PDIN (g/kg DM) ³	70	145	70	310
PDIE (g/kg DM) ⁴	74	118	95	230

¹ 70% barley, 22% maize and 8% oats. ² UFL, feed unit for lactation (Vermorel, 1989). ³ PDIN, true protein truly digestible in the small intestine when energy in the rumen is not limiting (Vérité and Peyraud, 1989).

⁴ PDIE, true protein truly digestible in the small intestine when degraded N in the rumen is not limiting (Vérité and Peyraud, 1989).

¹ 70 % d'orge, 22 % de maïs et 8 % d'avoine. ² UFL : unité fourragère lait (Vermorel, 1989). ³ PDIN : protéines digestibles dans l'intestin permises par l'azote (Vérité et Peyraud, 1989). ⁴ PDIE : protéines digestibles dans l'intestin permises par l'énergie (Vérité et Peyraud, 1989).

were fed hay at libitum and 3 kg/day of dehydrated lucerne and were complemented on average with 3.4 kg/day of cereal concentrate. Animals of group S received for the same milk yield, 2.5 kg of soybean meal substituted to 2.5 kg of cereal concentrate. The cheeses were manufactured using three different milks: milk from groups C and S animals and milk from group C animals with added urea (AU).

Milk sampling

The milks used for cheesemaking were collected from the two groups of cows four times during the last week of each experimental period. The milk samples were composed of equal quantities of the evening milk stored 12 h at 4°C and of the morning milk. Milk from C group animals was split in two samples and 0.25 g/L of urea was added to one of them.

Cheese manufacture

Reblochon cheese is an 'Appellation d'Origine Contrôlée' labeled cheese. It is a small size (500 g, 180 mm diam, 25 mm thick) semi-hard,

pressed cheese which is manufactured exclusively with full fat raw milk originating from Haute-Savoie and a few districts of Savoie.

The experimental pilot plant had two 150-L vats. The three milk types (C, S, AU) were processed each time with the same technology. Sixty-six L of milk were used in each vat, allowing the manufacture of 15 cheeses. During the two periods, 24 batches were made (eight repetitions of the three treatments) as described previously (Martin et al, 1997). A culture of mixed thermophilic freeze-dried starters (FYS11, Texel, Dangé-St-Romain, France) and rennet-containing 520 mg active chymosin/L were used for cheesemaking.

Analyses were carried out on the ripened cheeses when they were 30 days old.

Measurements and analyses

Milk analyses

Milk samples were collected from each vat before addition of the starter culture. The tests of clotting ability and separation of the soluble and curd phases of the milk were conducted 1 h after sampling. The milk samples were stored at 4°C and

analyzed the same day. The milk fat, protein, casein, total and soluble calcium, somatic cell count, total bacteriological and coliform count, pH and clotting ability were analyzed as described previously (Martin et al, 1997). Phosphorus and urea contents were determined respectively by the complexometric (NF V04-284, 1985) and the dimethylamino-4 benzaldehyde (DMAB) methods.

Cheese physico-chemical analyses

Fresh cheese temperature and pH were measured upon moulding, 30 min, 1 h, 2 h and 3 h later and on demoulding (4 h). A whey sample was taken from the vat before moulding. pH, dry matter and fat content were determined after 30 min of pressing, on demoulding and 1, 6 and 30 days after.

Dry matter, fat, pH and cheese yield were determined as described previously (Martin et al, 1997). Total nitrogen (N), N soluble in water (soluble in tri-sodium citrate 0.5 mol/L at pH 4.6, WNS) and non protein N (soluble in 12% trichloroacetic acid, NPN) were analyzed using the Kjeldahl method.

Sensory analyses

One cheese from each batch was submitted for tasting to a panel of 12 trained subjects. A product profile previously established by the Institut Technique du Gruyère was used. The subjects of the panel were trained to assess the intensity of each of the attributes included in the profile using a structured scale from 0 to 6. The profile comprised five attributes for texture, six for taste and three for odor. The three samples of cheese manufactured the same day were coded and presented simultaneously to all tasters in a random order which was different for each person. During each session, six samples of cheeses corresponding to 2 days of manufacture were analyzed. Each person scored one attribute for the three cheeses presented simultaneously: first odor, then texture, then taste attributes and last hedonistic appreciation. The score for one attribute is the mean of the 12 panel scores.

Rheological analyses

Evaluation, of mechanical properties of cheeses were performed using a FIRA-NIRD extruder with a spring resistance of 3 kg. Samples were

analyzed after being conditioned for 1 h at $22^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The extrusion force was the force required to extrude the cheese sample.

Statistical analysis of results

The results were processed by analysis of variance (GLM procedure: SAS, 1987) whilst including in the model the period, the day in the period, the group and the treatment.

RESULTS

Milk and fresh cheese characteristics

Somatic cells, total bacterial and total coliform counts were always respectively lower than $150 \cdot 10^3/\text{mL}$, $50 \cdot 10^3 \text{ cfu}/\text{mL}$ and $100 \text{ cfu}/\text{mL}$ (table II). Milk urea content was significantly higher in group S compared to group C. Calcium and protein contents were slightly lower in group S. Fat, casein and P content were not modified. Milks of group S formed curd less readily than milks of group AU which themselves formed curd less readily than milks of group C, although pH remained unmodified. The high nitrogen level in the diet slightly decreased curd firmness measured 30 min after renneting. Concerning curd firmness, milks from AU groups were similar to those of group C. Curd firmness after a firming period equal to clotting time was not modified by the treatment.

In the vat, acidification kinetics were very similar for the three milks tested. In mould (1 h and 2 h) and at demoulding (3 h), S and AU cheeses were less acid than C cheeses ($P < 0.05$) (table III). At demoulding, C cheeses were also dryer although the differences were not statistically significant. One day later, pH was the same in the three treatments and dry matter was lower for the AU cheeses. During the period spent in the cellar (6 days), C cheeses were slightly more acid and dry matter of AU cheese remained

Table II. Effects of diets on milk characteristics.*Influence des régimes alimentaires sur les caractéristiques des laits.*

<i>Treatment</i>	<i>C milk</i>	<i>S milk</i>	<i>AU milk</i>	<i>RSD</i> ³
Number of observations	8	8	8	
Somatic cells count ($\times 10^3/\text{mL}$)	73	68	74	24
Total bacterial count ($\text{cfu} \times 10^3/\text{mL}$)	11	12	10	4
Total coliform count (cfu/mL)	36	12	15	45
Fat (g/L)	37.5	37.1	37.5	0.5
Proteins (g/L)	30.0	29.6	30.0	0.3
Casein (g/L)	24.0	23.8	23.9	0.2
Casein (% proteins)	79.9	80.1	79.7	0.6
Urea (g/L)	0.24 ^a	0.49 ^b	0.49 ^b	0.04
Ca (g/L)	1.14 ^{ab}	1.13 ^a	1.15 ^b	0.01
P (g/L)	0.75	0.79	0.77	0.13
pH	6.75	6.77	6.77	0.04
Rennet clotting time (min)	14.9 ^a	15.9 ^b	15.2 ^{ab}	0.7
Curd firming time (min)	10.0 ^a	11.3 ^b	10.7 ^c	0.5
Curd firmness ¹ (mm)	27.5 ^a	23.6 ^b	26.3 ^a	1.3
Curd firmness ² (mm)	27.3	26.7	26.6	1.1

¹ 30 min after rennetting. ² After a firming period equal to clotting time. ³ Residual standard deviation. ^{a, b, c} Values with different superscripts within the same row significantly differ ($P < 0.05$).

¹ Trente minutes après l'emprésurage. ² Après une durée de raffermissement égale au temps de prise. ³ Écart type résiduel. ^{a, b, c} Les valeurs d'une même ligne suivies de lettres différentes sont significativement différentes ($p < 0,05$).

lower. No differences were observed for the Ca and NaCl content.

When composition was highly similar except for urea content which was higher for AU and S groups.

Matured cheese characteristics

Chemical composition and cheese yields

The chemical composition of matured cheese made from groups S and AU milks differed in certain aspects from cheese made from group C milks (table IV). The former were less dry and less fatty. Dry matter and fat content of groups AU and S cheese were

similar. AU cheeses were more salty and had a slightly lower Ca content and a slightly higher NH_3 content although differences were not statistically significant. Matured cheese yields were slightly lower for group S.

Sensory properties

The greatest differences between treatments were found in the physical and sensory properties of cheeses (table V). Considering the texture attributes, cheeses of groups AU and S were highly different from cheeses of group C. The force needed to extrude cheese of group C was 20% lower than that needed to extrude cheese from groups S and AU. In the mouth, cheeses of group C had a

Table III. Cheesemaking parameters and fresh cheese characteristics.*Paramètres de fabrication et caractéristiques des fromages frais.*

<i>Treatment</i>	<i>C milk</i>	<i>S milk</i>	<i>AU milk</i>	<i>RSD</i> ¹
Number of observations	8	8	8	
Whey composition				
Proteins (g/kg)	6.8	6.7	6.7	0.1
Fat (g/kg)	11.0	10.5	10.3	1.4
Urea (g/L)	0.22 ^a	0.42 ^b	0.43 ^b	0.01
Ca (g/L)	0.37	0.38	0.39	0.02
Fresh cheese acidification characteristics				
pH (1 h)	6.25 ^a	6.34 ^b	6.32 ^b	0.04
pH (2 h)	6.09 ^a	6.24 ^b	6.21 ^b	0.10
pH (3 h)	5.85 ^a	5.99 ^b	6.00 ^b	0.14
pH (1 day)	5.24	5.24	5.26	0.07
pH (6 days)	5.02 ^a	5.08 ^b	5.08 ^b	0.05
Dry matter 3 h (%)	44.4	43.2	43.0	1.7
Moisture/non-fat dry matter ² (3 h)	2.43	2.56	2.59	0.18
Dry matter 1 day (%)	45.2	45.5	44.6	1.5
Moisture/non-fat dry matter ² (1 day)	2.38	2.35	2.43	0.20
Dry matter 6 days (%)	48.0 ^a	48.2 ^a	46.9 ^b	1.2
Ca 6 days (mg/100 g)	564	563	556	38
NaCl 6 days (%)	1.45	1.36	1.35	0.2

¹ Residual standard deviation. ² Moisture/non-fat dry matter = (100 - dry matter) / (dry matter - fat). ^{a, b, c} Values with different superscripts within the same row significantly differ ($P < 0.05$).

¹ Écart type résiduel. ² Humidité / extrait sec dégraissé = (100 - extrait sec) / (extrait sec - matière grasse). ^{a, b, c} Les valeurs d'une même ligne suivies de lettres différentes sont significativement différentes ($p < 0,05$).

firmer, a more chalky and a less creamy texture. Compared to cheeses of group S, the texture of cheeses of group C was also more pasty and less homogeneous. Textures of group S and AU cheeses were similar although cheeses of group AU were slightly more pasty and chalky.

Considering most of the taste attributes, cheeses of group S were different from cheeses of groups AU and C. Cheeses of groups AU and C were more acid, slightly more bitter and taste was more intense. Nevertheless, sweet taste was scored lower for cheeses of the group C as compared to

cheeses of groups S and AU. No differences were found for the smell attributes.

DISCUSSION

Effect of high nitrogen supply in the diet on milk characteristics

The large increase in milk urea content observed with high nitrogen supply in the diet was close to the one expected considering the predictive milk urea content formula proposed by Vérité et al (1995). In this

Table IV. Chemical composition of ripened cheeses (30 days).*Composition chimique des fromages affinés (30 jours).*

<i>Treatment</i>	<i>C milk</i>	<i>S milk</i>	<i>AU milk</i>	<i>RSD¹</i>
Number of observations	8	8	8	
Analyses				
pH	5.87	5.86	5.98	0.10
Dry matter (%)	47.9 ^a	46.5 ^b	46.1 ^b	1.29
Fat (%)	25.1 ^a	23.8 ^b	23.7 ^b	0.8
Fat in dry matter (%)	52.4	51.3	51.4	1.2
Moisture/non-fat dry matter ²	2.28	2.38	2.40	0.14
Ca (mg/100 g)	401	400	372	38
NaCl (%)	1.47 ^a	1.45 ^a	1.62 ^b	0.09
TN ³ (g/100 g)	19.9	20.1	19.7	0.8
WSN ⁴ (g/100 g)	4.01	3.95	3.99	0.19
NPN ⁵ (g/100 g)	2.10	2.04	1.98	0.14
NH ₃ (mg/100 g)	176	178	196	26
Corrected matured cheese yield (%)	11.35 ^a	11.05 ^b	11.21 ^{ab}	0.23

¹ Residual standard deviation. ² Moisture/non-fat dry matter = (100 - dry matter) / (dry matter - fat). ³ Total nitrogen. ⁴ Soluble nitrogen. ⁵ Non protein nitrogen. ^{a, b, c} Values with different superscripts within the same row significantly differ ($P < 0.05$).

¹ *Écart type résiduel*. ² *Humidité / extrait sec dégraissé = (100 - extrait sec) / (extrait sec - matière grasse)*. ³ *Azote total*. ⁴ *Azote soluble*. ⁵ *Azote non protéique*. ^{a, b, c} *Les valeurs d'une même ligne suivies de lettres différentes sont significativement différentes (p < 0,05)*.

trial, the increase of milk urea content, obtained by feeding or by adding urea into the milk was not responsible for any dissociation of casein or Ca into the soluble phase as observed by Ali et al (1980) when adding greater quantities of urea. Coagulating properties of milks were only slightly damaged by increasing milk urea content, as observed by most of the authors (Millet, 1989; Guinot-Thomas, 1992; Mariani et al, 1992) and the effect of urea was of no practical relevance. The slight decrease in milk protein content observed with the high nitrogen supply is surprising because milk protein content depends mainly on the energy supply (Coulon and Rémond, 1991) which was similar for the two diets and because nitrogen supply should not influence milk protein

content (Rémond, 1985). In this experiment, the low protein contents of milks were mainly due to the early stage of lactation of the cows (Schutz et al, 1990).

Effect of milk urea content on matured cheese characteristics

The main effect of milk urea, whether it originated from addition to milk or from a high N diet, concerned ripened cheese texture which was less dry, less firm and chalky and more creamy. This confirms the findings of Ali et al (1980) who showed that addition of urea to milk leads to moister cheeses. As observed by Millet (1989), the poor draining ability of the high urea content

Table V. Sensory characteristics of ripened cheeses (30 days).
Caractéristiques sensorielles des fromages affinés (30 jours).

<i>Treatment</i>	<i>C milk</i>	<i>S milk</i>	<i>AU milk</i>	<i>RSD¹</i>
Number of observations	8	8	8	
Extrusion force (N)	11.4 ^a	9.1 ^b	8.9 ^b	1.1
Sensory properties (6)				
Homogenous texture	2.65 ^a	3.67 ^b	2.82 ^{ab}	0.91
Firm texture	3.93 ^a	2.35 ^b	2.71 ^b	0.66
Creamy texture	1.91 ^a	3.25 ^b	2.92 ^b	0.69
Pasty texture	3.35 ^a	2.54 ^b	3.23 ^a	0.39
Chalky texture	3.10 ^a	1.42 ^b	2.28 ^c	0.56
Intense taste	3.55 ^a	3.24 ^b	3.69 ^a	0.38
Acid taste	2.99 ^a	2.25 ^b	2.76 ^a	0.40
Bitter taste	2.58 ^a	1.99 ^b	2.37 ^{ab}	0.44
Milk taste	1.90	2.13	2.08	0.23
Salty taste	2.69	2.43	2.72	0.28
Sweet taste	1.83 ^a	2.41 ^b	2.18 ^b	0.37
Sharp odor	2.26	2.07	2.37	0.38
Sweet odor	2.38	2.44	2.19	0.31
Milk odor	2.50	2.23	2.36	0.31

¹ Residual standard deviation. ^{a, b, c} Values with different superscripts within the same row significantly differ ($P < 0.05$).

¹ *Écart type résiduel.* ^{a, b, c} Les valeurs d'une même ligne suivies de lettres différentes sont significativement différentes ($p < 0,05$).

milks may be partly explained by their lower acidification rate in mould, resulting in moister cheeses at demoulding. Urea, whether it comes from addition to milk or from a high N diet, seemed to slow down acidification. Such an effect of urea has already been observed by some authors who suggested that urea inhibits activity of yogurt starter culture and as a consequence acid development by contaminating micro-organisms (Podhorsky and Cvak, 1989). Further, some streptococci can convert urea to $\text{CO}_2 + \text{NH}_3$ (Juillard et al, 1988) which could explain partly the slower acidifica-

tion observed in this trial with high urea content, streptococci being the main micro-organisms of the starters involved in early acidification of Reblochon cheese. The effect of milk urea on texture can not be explained by a more intense proteolysis as no differences were observed on WSN/NT and NPN/NT ratios.

From a taste point of view, most differences were found between cheeses of group S on the one hand and cheeses of groups C and AU on the other hand, except for the sweet taste which was lower for the group C cheeses. This is probably linked to the tex-

ture which was firmer for C cheeses. Indeed, we observed that sweet taste was linked to firm, pasty and chalky texture (respectively, $r = -0.46, -0.54$ and $-0.51, P < 0.05$). Interactions between texture and taste are indeed very often observed (Lawrence et al, 1984). The other differences in cheese taste were not linked to the level of proteolysis; no differences were found in the cheese nitrogen fractions, unlike the results of Millet (1989) who found a more intense proteolysis when urea was added to milk. Nevertheless, these results are difficult to compare because urea was added in much greater quantities by Millet (1989) (1 g/L) than for this trial (0.25 g/L). As only group S cheeses were different from the others, differences in taste were probably not linked directly to urea concentration in milk. Considering the influence of urea on moisture content and on texture of matured cheese, this result is surprising. Nevertheless, it is possible that the observed disparities between cheeses of groups C and AU on the one hand and cheeses of group S on the other hand were due to other components of milk modified by the high nitrogen supply in the diet or by the nature of the concentrate used (soybean meal vs cereals). Indeed, some authors have shown that feeding can influence cheese characteristics (Urbach, 1990; Grummer, 1991; Verdier et al, 1995; Grappin and Coulon, 1996). However, this study does not enable us to verify these hypotheses.

CONCLUSION

This trial shows that nitrogen supply in the diet can influence cheesemaking and matured cheese chemical composition, texture and taste. Milk urea is directly involved in acidification kinetics and differences of texture. As milk urea content depends on feeding practices, seasonal variations of milk urea content can explain some of the associations observed by Martin and Coulon

(1995) between feeding practices and matured cheese characteristics (turning out to pasture, hay quality in winter or grass phenological stage in summer). The results suggest that it would be interesting for the cheesemakers to determine urea content of milk in some particular periods of the year in order to choose their cheesemaking parameters. It would be of interest now to generalize these observations to other cheese technologies and to verify if it is possible to correct the influence of milk urea by modifying cheesemaking parameters.

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