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Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk

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Abstract

The effects of separate infection with four major pathogens frequently associated with the occurrence of subclinical mastitis in cows (*Staphylococcus aureus*, *S. chromogenes*, *Escherichia coli* and *Streptococcus dysgalactiae*) on milk quality for cheese production were studied for quarters of the same animal. Infection increased somatic cell count (SCC), modified leucocyte distribution, decreased lactose concentration and increased proteolysis of casein. Regardless of bacteria type, the plasmin activity in milk from the infected glands increased ~2 fold compared with uninfected quarters. These changes were associated with increased rennet clotting time and decreased curd firmness for milk from infected glands, indicating that milk quality for cheese production was negatively affected by infection. Although the general pattern of bacterial invasion was similar, each type of bacteria elicited the above-described responses in a specific manner. SCC, commonly used by the dairy industry as a measure of milk hygienic quality, provided the poorest prediction of milk quality for cheese production in comparison to indices of proteolysis of casein.
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Keywords: Udder infection; Somatic cell count; Plasmin; Curd firmness

1. Introduction

Udder health affects the yield and quality of milk and, consequently, cheese production and quality. In milk with high somatic cell count (SCC), the deterioration of cheese-making properties occurs, i.e., a longer rennet coagulation time and a weak coagulum, which in turn leads to increased moisture content in the cheese and an overall lower cheese yield (Auldist, Coats, Sutherland, Mayes, & McDowell, 1996; Auldist & Hubble, 1998; Barbano, Rasmussen, & Lynch, 1991; Klei et al., 1998; O'Brien, Meaney, McDonagh, & Kelly, 2001; Rogers & Mitchell, 1994). These negative correlations have also been found for milk from sheep and goats (Galina, Morales, Lopez, & Carmona, 1996; Leitner, Chaffer et al., 2004; Leitner, Merin, Silanikove et al., 2004). However, the relationship between bacterial infection, inflammatory response, proteolysis of casein and, consequently, cheese yield and quality are complicated (Le Roux, Laurent, & Moussaoui, 2003). Thus, it is doubtful if SCC or their partitioning can be used as a single reliable measure to correlate between intramammary infection (IMI) and cheese yield and quality.

Milk from mastitic udders exhibits increased proteolytic activity (Auldist et al., 1996; Le Roux, Colin, & Laurent, 1995; Le Roux, Girardet, Humbert, Laurent, & Linden, 1995; Schaar & Funke, 1986; Urech, Puhan, & Schalibaum, 1999). Plasmin (PL) is the most important protease in milk from both healthy udders and udders with elevated SCC, but the non-plasmin proteases become more important with increasing severity of udder inflammation (O'Farrell, Sheehan, Wilkinson, Harrington, & Kelly, 2002; Verdi & Barbano, 1988; Verdi, Barbano, Dellavalle, & Senyk, 1987). Proteolysis of casein leads to a decrease in the

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relative proportion of caseins (CNs) (especially β-CN and α_{s1}-CN) with simultaneous clear increased levels of γ-CNs and proteose peptones (Auldist et al., 1996; Barbano et al., 1991; Le Roux, Colin et al., 1995; Le Roux, Girardet et al., 1995; Urech et al., 1999).

The invasion of bacteria into the lumen of the mammary gland and their accumulation above a given number induce the action of the innate mammary gland immune defense system, which may generally be defined as an inflammatory response. The inflammatory response may be localized to the mammary gland and may not include clinical signs such as edema and redness of the gland and in such cases is defined as subclinical (Harmon, 1995). Bacterial infection may affect proteolysis of casein and micelle properties by two main routes. Firstly, by secreting extracellular enzymes and promoting enzyme activity, different bacteria may directly cause different "types" of physico-chemical damage to the milk (Leigh & Lincoln, 1997; Zavizion, White, & Bramley, 1997). Secondly, through activation of the host innate immune system, milk from udders infected with different types of bacteria but with similar SCC may have different characteristics owing to different leucocyte populations and leucocyte-associated proteases (Coulon et al., 2002). Finally, a combination of the two mechanisms outlined may be significant. It should be noted that the interaction between bacteria and the host system is species-specific. The types of bacteria present during infection invoke unique immune responses and thus differently alter milk quantity and quality (Le Roux et al., 2003). The bacterial effects on the immune response and consequential changes in milk yield and composition are notable in the case of clinical mastitis (Coulon et al., 2002; Hamman & Kromker, 1997). However, the work of Coulon et al. (2002) is the sole source of concise information on the effect of bacteria type and consequential changes in milk yield and composition in the case of sub-clinical mastitis. Sub-clinical mastitis is the main form of mastitis in modern dairy herds, exceeding 20–50% of cows in given herds (Janosi & Baltay, 2004; Pitkala, Haveri, Pyorala, Myllys, & Honkanen-Buzalski, 2004; Wilson, Gonzalez, & Das, 1997).

In the present study, the relationships between sub-clinical bacterial infection, milk composition, activity of the plasmin, proteolysis of CN and curd firmness were studied in order to understand better the causes for inferior cheese yield and quality under such conditions. The effect of four major udder pathogens: *Staphylococcus aureus*, *S. chromogenes*, *Escherichia coli* and *Streptococcus dysgalactiae* which are commonly associated with sub-clinical mastitis, were examined.

2. Materials and methods

2.1. Animals

Thirty three Israeli-Holstein dairy cows, in which one quarter or more were chronically infected with subclinical mastitis by one of the following bacteria: *S. aureus*, *E. coli*, *Str. dysgalactiae* or *S. chromogenes* were included in the study. All infected quarters were monitored for its bacterial condition for 2–3 months prior to the beginning of the study. Confirmation of bacteria identity was based on aseptic quarter foremilk samples taken 3 times over 2 days intervals and sent to the laboratory for analysis within 1 h (IDF, 1985). The chosen cows were in mid- to late-lactation, yielding between 28 to 52 L day^{-1}. The cows were milked thrice daily (5:00, 12:00 and 20:00) and fed a typical Israeli total mixed ration comprised of 65% concentrate (17% protein) and 35% forage.

2.2. Sample analysis

Milk was sampled from each quarter during the morning milking. For the bacteriological tests the teats were cleaned and disinfected and 5 mL of milk was sampled. An additional sample (200 mL) from about 1 L of milk was taken from each quarter for analyses as follows: SCC (Coulter cell counter—Z1, Coulter Electronics Limited, Luton, England) and differentiation of cells by FACS Calibur flow cytometer (Becton-Dickinson Immunocytometry System, San Jose, CA) (Leitner, Eligulashvily, Krifucks, Perl, & Saran, 2003) using anti-bovine monoclonal antibodies (VMRD Inc., Pullman, WA, USA). Monoclonal antibodies were: anti-CD18/11a—BAT 75A (IgG-1), anti-CD4—GC 50A1 138A (IgM), anti-CD8—CACT 80C (IgG-1), anti-CD21—BAQ 15A (IgM), anti-CD14—CAM 36A (IgG-1), anti-polymorphonuclear (PMN) (G1) (IgM). All monoclonal antibodies used were species-reactive with bovine cells. The secondary polyclonal antibodies (CALTAG Laboratories, Burlingame, CA, USA) used were: goat anti-mouse IgG-1 conjugated with TRI-COLOR (TC) and goat anti-mouse IgM conjugated with FITC. Milk gross composition (protein, fat and lactose contents) was measured using the Milkoscan 6000, and SCC was measured with a Fossomatic 360 (Foss Electric, Hilleröd, Denmark). Casein content was determined according to standard methods (Marshall, 1992). Whey proteins were analyzed according to the method described by Shamay, Shapiro, Barash, Bruckental, and Silanikove (2000). Proteose-peptones (p-p) were isolated according to the method of Andrews (1983) by boiling skim milk and precipitation of non-p-p proteins by acidification with HCl 5 N to pH 4.6 and centrifugation. The p-p concentration was determined in this fraction by the Bradford method, which will result in values somewhat lower than those obtained by the Kjeldahl method (Le Roux, Colin et al., 1995). Plasmin activator (PA), plasminogen (PLG) and PL activities were determined according to the method described by Silanikove, Shamay, Sinder, and Moran (2000).

2.3. Microbiological examinations

Microbiological analysis was performed according to accepted standards (Hogan et al., 1999). From every milk

sample, 0.01 mL was spread onto blood-agar plates (Bacto-Agar; Difco Laboratory Becton, Dickinson and Company, France) containing 5% of washed red sheep blood cells and on MacConkey plates. All plates were incubated at 37 °C and examined for growth after 18 and 42 h. Colonies suspected to be staphylococci were tested for coagulase (tube test, Anilab, Rehovot, Israel). Strain identification was carried out with the API STAPH-IDENT, 32 Staph kit or rapid ID 32 STREP (bioMerieux S.A., 69280 Marcy-l'Etoile, France). When the percentage of micrococci-like bacteria that matched the test strain exceeded 90%, the strain was regarded as specific.

2.4. Curd firmness and clotting time

Curd yield and clotting time (Tc) were determined by the Optigraph© (Ysebaert, Frepillon, France). Samples (10 mL) were placed in the wells and equilibrated at 30 °C. Fromase 15 TL (Gist-Brocades nv, Delft, The Netherlands) was diluted to achieve clotting at about 600 s. The instrument was set to measure Tc in seconds and curd firmness in volts, 30 min after enzyme addition (A30). All samples were analysed in triplicate.

2.5. Statistical analysis

Data (SCC, log SCC, PMN, CD4, CD8, CD14, whey proteins, p-p, PLG, PA, PL, Tc, A30 and milk composition: fat, protein, CN and lactose) were analyzed according to a model that takes into account the cow and the quarter infection status uninfected, *S. aureus*, *E. coli*, *Str. dysgalactiae*, or *S. chromogenes*, applying a two-way ANOVA design using the following linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk},$$

where μ is the grand mean; α_i is the effect of the ith cow; β_j is the effect of the jth infection status; and e_{ij} represents the residual between-quarters error. Comparisons between infection group's pairs were made by *t*-test using the Tukey-Kramer HSD.

In addition, a correlation matrix between milk log SCC, protein, CN, fat, lactose, whey proteins, p-p and PL as independent variables and Tc or curd firmness as depended variables were determined. All statistical analyses were carried out with JMP software (SAS Institute, 2000).

3. Results

SCC were significantly higher in the infected quarters than in the uninfected ones, regardless of bacteria spices (Table 1). Mean SCCs were as follows: *E. coli* and *Strep. dysgalactiae* ~4×10^6 cells mL^{-1}, *S. aureus* ~1×10^6 cells mL^{-1}, *S. chromogenes* ~5×10^5 cells mL^{-1} and in the milk samples of the uninfected quarters 1×10^5 cells mL^{-1}. The proportion of PMN as of total SCC increased significantly in all the infected quarters in comparison with the uninfected ones. No significant difference was found in the proportion of CD4$^+$- or CD8$^+$-bearing T-lymphocytes or CD14$^+$. However, the absolute number of lymphocytes and macrophages increased significantly compared to the uninfected glands and varied significantly between the different bacteria species (Table 1).

In general, bacterial infection did not significantly affect the concentration of fat and protein in milk. However, whey protein concentrations were significantly higher in milk from the quarters infected with *Strep. dysgalactiae* or *E. coli* and their percentage of total protein was ~20% in the uninfected and ~30% in the infected quarters, respectively. The concentration of p-p in infected quarters was significantly higher than in the uninfected once. The ratio of whey protein:CN was 0.25 in the uninfected quarters and ranged between 0.28 and 0.42 in the infected ones; similarly the ratio of p-p:CN was 2.04 in the uninfected quarters compared with 2.28–4.84 in the infected ones (Table 2). The lactose concentration in milk was lower in all the infected quarters in comparison to the

Table 1
SCC, log SCC and distributions of PMN, lymphocytes bearing CD4$^+$ or CD8$^+$ T-lymphocytes and macrophages (CD14$^+$) (absolute number in parentheses) in 1 mL of milk from 33 cows, as affected by intra-mammary bacterial infections of quarters[a]

Bacteria[b]	SCC (×1000)[c]	Log SCC	PMN (%)	CD4$^+$ (%)	CD8$^+$ (%)	CD14$^+$ (%)
Uninfected (33)	116±20[c]	4.93±0.08[b]	29±3.3[c] (34)[c]	3.1±0.9 (5)[c]	5.7±1.6 (7)[c]	5.5±1.8 (6)[c]
Streptococcus dysgalactiae (23)	3146±324[a]	6.36±0.09[a]	57±7[a,b] (1793)[a]	3.3±1.1 (104)[a]	10.5±2.0 (330)[a]	9.8±2.2 (308)[a]
Staphylococcus aureus (9)	865±727[b]	6.23±0.21[a]	71±1.7[a] (614)[b]	3.1±4.0 (27)[b]	2.7±7.1 (23)[b]	2.5±7.9 (22)[c]
Staphylococcus chromogenes (11)	543±129[c]	5.41±0.16[b]	54±8.5[a,b] (293)[b]	4.0±2.4 (22)[b]	9.6±4.4 (52)[b]	9.9±4.8 (54)[a,b]
Escherichia coli (3)	3960±793[a]	6.42±0.23[a]	75±9.1[a] (2970)[a]	1.8±2.2 (71)[a]	4.4±4.0 (174)[a,b]	12.6±4.4 (499)[a]

[a]Values shown are LSMeans±SE.
[b]Numbers of quarters analysed shown in brackets.
[c]Somatic cells counted with Fossomatic 360.

Table 2
Levels of fat, protein, casein, whey protein, proteose-peptone, lactose, plasminogen, plasmin activator and plasmin from 33 cows as affected by intra mammary bacteria infections of quarters[a]

Parameter	Uninfected (33)	*Strep. dysgalactiae* (23)	*S. aureus* (9)	*S. chromogenes* (11)	*E. coli* (3)	*P* (F)
Fat ($g L^{-1}$)	31.2±0.3	33.8±0.8	34.2±0.8	33.8±0	29.8±0.8	NS
Protein ($g L^{-1}$)	32.0±0.8	33.0±1.1	33.7±2.3	33.0±1.6	40.3±2.3	NS
Casein ($g L^{-1}$)	26.2±0.6	24.6±0.8	26.8±1.7	25.6±1.2	27.4±1.7	NS
Whey protein ($g L^{-1}$) (%)	6.60 ± 0.53^{b} (20.6)	9.12 ± 0.70^{a} (27.6)	$7.47\pm1.53^{a,b}$ (22.2)	$7.46\pm1.08^{a,b}$ (22.69)	12.55 ± 1.53^{a} (31.1)	0.0041
Proteose-peptone ($\mu g mL^{-1}$)	490 ± 62^{b}	1043 ± 84^{a}	$743\pm219^{a,b}$	563 ± 108^{b}	$1374\pm314^{a,b}$	0.0002
Whey/casein	0.25 ± 0.02^{b}	0.39 ± 0.03^{a}	$0.28\pm0.07^{a,b}$	$0.28\pm0.05^{a,b}$	$0.42\pm0.07^{a,b}$	0.0068
Proteose-peptone/casein (×100)	2.04 ± 0.23^{b}	4.62 ± 0.34^{a}	$2.94\pm0.51^{a,b}$	2.28 ± 0.37^{b}	$4.84\pm1.05^{a,b}$	0.0001
Lactose (mM)	144 ± 3^{a}	123 ± 4^{b}	$136\pm7^{a,b}$	$140\pm6^{a,b}$	$124\pm17^{a,b}$	0.0091
Plasmin ($U^{**} mL^{-1}$)	10.9 ± 2.3^{b}	24.8 ± 3.1^{a}	$19.2\pm4.8^{a,b}$	$15.4\pm4.0^{a,b}$	$20.1\pm11.7^{a,b}$	0.0095
Plasminogen ($U^{**} mL^{-1}$)	149±8	143±79.1	137±46.0	154±15	288±45	NS
Plasminogen activator ($U^{**} mL^{-1}$)	532±13	538±18	510±29	536±24	578±69	NS

Means within a line with no common superscript differ significantly ($P<0.05$).
[a]Values shown are LSMeans±SE. NS = not significant. U = activity unit; 1 unit is the amount of PL that produces a change in absorbance of 0.1 at 405 nm in 60 min.

Table 3
Values for clotting time and curd firmness 20 (A20) or 30 (A30) min after rennet addition to milk from 33 cows as affected by intra-mammary bacterial infections of quarters[a]

Bacteria[b]	Clotting time (s)	Curd firmness (V)	
		A20	A30
Uninfected (33)	650 ± 300^{c}	4.13 ± 0.97^{a}	6.58 ± 1.17^{a}
Streptococcus dysgalactiae (23)	2490 ± 1633^{a}	0.41 ± 0.80^{c}	1.02 ± 1.49^{c}
Staphylococcus aureus (9)	1078 ± 578^{b}	1.23 ± 0.91^{b}	3.22 ± 1.35^{b}
Staphylococcus chromogenes (11)	1225 ± 1544^{b}	1.78 ± 2.2^{b}	3.81 ± 2.80^{b}

Means within a column with no common superscript differed significantly ($P<0.05$).
[a]Values shown are LSMeans±SE.
[b]Numbers of quarters analysed in brackets.

uninfected ones but it was significantly different from uninfected quarters only with *Str. dysgalactiae* and *E. coli*.

The PLG level and PA activity did not differ significantly between infected and the uninfected quarters, except for a sharp increase of PLG level in the milk of quarters infected with *E. coli* (Table 2). In contrast, the activity of PL increased ~2 fold in the milk compared with the uninfected quarters, regardless of bacteria type, although not all the differences were significant.

The rennet clotting time of milk from the infected quarters (1078–2490 s) was considerably longer than in the uninfected quarters (650 s). In parallel, curd firmness measured either at 20 or 30 min after enzyme addition was significantly lower in the infected quarters (Table 3) than in controls.

4. Discussion

4.1. Interaction between bacteria type and the innate immune system

In bovine milk from uninfected glands, macrophages (Concha, Holmberg, & Astrom, 1986) or epithelial cells (Leitner, Shoshani, Krifucks, Chaffer, & Saran, 2000) represent the predominant cell type (35–79%). Following detection of pathogen invasion into the mammary gland, the macrophages and epithelial cells release chemoattractants, which trigger migration of leucocytes, mainly PMNs from the blood toward the infection site, increasing their proportion from basal level of 5–25% to approximately 90% of total cells (Concha et al., 1986; Leitner et al., 2000). In the process of phagocytosis and killing of invading bacteria by the PMN and macrophages nitrite, oxygen species and a range of proteolytic enzymes are released (Paape, Bannerman, Zhao, & Lee, 2003). When the infection progresses to the chronic stage, overall the type and the proportion of the leucocytes fluctuate with decreased proportion of PMNs and increase of $CD8^{+}$-bearing T-lymphocytes, however, the proportion as well as number of the different types of leucocytes is bacteria specific (Leitner et al., 2000). In the present study all the cows were in a chronic stage of subclinical mastitis caused by various bacteria, which elicited an increase in SCC. Although there was no significant difference in the proportions of the leucocytes types, their absolute number (proportion times SCC) was bacteria-specific.

4.2. Interaction between bacteria type and milk composition

The increase in whey protein concentration and in indices of proteolysis of CN (i.e., ratios of whey protein/CN and p-p/CN) indicate that CN undergoes extensive degradation in milk coming from infected glands. Despite the latter, no significant difference was found in total CN concentration between uninfected and infected glands. Thus, the lack of response of total CN concentration in milk from infected glands is likely related to activation of the plasmin system resulting in partial CN degradation, and in turn its consequence on milk yield and composition as found in our studies with sheep and goats (Leitner, Chaffer et al., 2004; Leitner, Merin, & Silanikove, 2004). Enzymatic hydrolysis of CN liberates peptides that serve as local regulators of mammary gland function (Shamay, Leitner, Shapiro, & Silanikove, 2003; Shamay, Mabjeesh, & Silanikove, 2002; Silanikove et al., 2000). A peptide derived from the activity of PL on β-CN (β-CN 1-28) down-regulates milk secretion in cows and goats; its activity was correlated with its ability to block potassium channels in the apical membranes of mammary epithelia (Silanikove et al., 2000). This peptide reduces the output of lactose and other osmotic components from the alveoli into the gland lumen (Silanikove et al., 2000), as also found in the present study. When the increase in PL activity is high (increase of 150% or more), as is the case during milk stasis or inflammation, hydrolysis of CN in goats (Shamay et al., 2002) and cows (Shamay et al., 2003) induces rapid drying-off of mammary secretions, which is associated with lower secretion of fat and protein.

4.3. Interaction between bacteria type and the plasmin system

The almost two-fold increase in PL activity in the milk of infected glands in comparison to its activity in uninfected glands found in the present study is consistent with previous results showing activation of the PL system during mastitic infection in cows (Moussaoui, Michelutti, Le Roux, & Laurent, 2002) and sheep and goats (Leitner, Chaffer et al., 2004; Leitner, Merin, Silanikove et al., 2004; Leitner, Merin, & Silanikove, 2004). The classical causes for accelerated PL activity in mammary secretion, namely, an increase in tissue-PA and/or rapid conversion of PLG to PL (Leitner, Chaffer et al., 2004; Leitner, Merin, & Silanikove, 2004; Politis, 1996) could not explain the increase in PL activity in the present study. Heegaard et al. (1994) and Zachos, Politis, Gorewit, and Barbano (1992) found a large increase in PA activity in mastitic cows in correlation with an influx of PMNs. However, the leucocyte-related PA activity (mostly urokinase-type PA) was not detected in the present experiments because most of the leucocytes were discarded during the preparation of the samples and because urokinase-type PA is closely associated to the leucocytes through urokinase-type PA receptor (Politis, Bizelis, & Rogdakis, 2002; Politis, Zavizion, Cheli, & Baldi, 2002). In addition, PA activity may be derived from or enhanced by infecting bacteria (Hayes, Larson, & Nielsen, 2005; Ward et al., 2004). If non-tissue-PAs were responsible for accelerated conversion of PLG to PL, an increase in influx of PLG to the gland could still remain undetected because of no change in the final content of PLG. Thus, further studies are needed to elucidate the cause for the increased PL activity during sub-clinical infection in bovines.

4.4. Interaction between bacteria type, proteolysis of casein and milk quality for cheese production

All types of bacteria tested in the present experiment, including *S. chromogenes*, which is still considered by many veterinarians as non-harmful environmental bacteria, elicited a marked deterioration in curd yield and firmness in milk withdrawn from infected glands. The deterioration in milk quality was bacteria-specific and, in the case of *Str. dysgalactiae,* the effect was so dramatic that almost no curd was produced from glands infected with these bacteria. This result and the poor quality of the curd strongly suggest that the damage caused to the CN micelle as a consequence of the bacterial infection was irreversible. Thus, processing milk coming from quarters infected with *Str. dysgalactiae* may reduce cheese yield in direct proportion to the volume of milk coming from such infected glands. A very high proportion of cows producing non-coagulating milk (13% of the samples) was reported in a recent publication from Finland (Ikonen, Morri, Tyriseva, Ruottinen, & Ojala, 2003), without addressing the potential cause for this phenomenon. Our data suggest that this may be related to high proportion of sub-clinically infected quarters in the sampled milk and that, over all, poor quality milk from sub-clinically infected cows (in one quarter or more) is a major problem for the dairy industry in relation to cheese production.

Today, SCC serves as the main criterion for grading milk in term of its hygienic quality. However, as shown in Table 4, among the parameters compared, log SCC had the

Table 4
Correlations between log SCC, contents of protein, casein, fat, lactose, whey proteins, proteose-peptone and plasmin and clotting time or curd firmness (A30) of milk from quarters infected with different bacteria

Parameters	Clotting time		Curd firmness (A30)	
	R	*P*-value	*R*	*P*-value
Log SCC	0.48	0.02	−0.46	0.001
Protein	0.51	NS[a]	−0.46	NS[a]
Casein	0.01	NS[a]	0.08	NS[a]
Fat	0.30	NS[a]	−0.18	NS[a]
Lactose	−0.64	0.002	0.61	0.003
Whey proteins	0.83	0.001	−0.72	0.001
Proteose-peptone	0.59	0.005	−0.60	0.004
Plasmin	0.65	0.001	−0.65	0.001

[a]NS = not significant.

lowest predictive value in regard to predicting milk quality for cheese production. Moreover, the major milk constituents, fat, protein and CN, were not significantly correlated with the curd forming properties, while lactose, whey proteins and p-p significantly correlated with the latter as was previously noted by Le Roux, Colin et al. (1995). Similarly, PL also significantly correlated with Tc and curd firmness. Despite the good correlation of the various degradation parameters with curd forming properties, the differences found between *Str. dysgalactiae* and *S. aureus* with similar SCC on curd firmness (Fig. 1) suggest that there are bacteria-specific interactions with the innate immune system. A probable explanation for the better prediction of proteolysis of CN indices (including whey proteins) relates to the specific interaction between bacteria type and the immune system and consequently the induction of uncharacterized, probably enzymatic and oxidative responses, which augment proteolysis of CN. The proportion of whey proteins and indices of proteolysis of CN (whey/CN, p-p/CN) explained only part of the variability suggesting the involvement of bacterial, bacterial proteases and/or other indigenous enzymes, which are multiple and complex (Fig. 2). Additional factors such as changes in CN-micelles morphology and structure will be needed to be included in predictive models to increase their effectiveness. Therefore, at present, perhaps the most practical means to evaluate milk quality for cheese yield is by direct measurements of milk clotting time and curd firmness by optical or comparable means, which provides rapid and direct relevant results and best prediction for commercial cheese making.

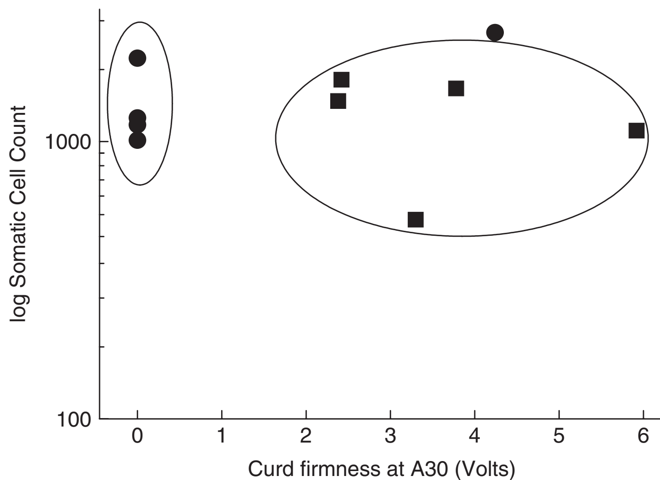

Fig. 1. Effect of infection by two bacteria species (●, *Streptococcus dysgalactiae*; ■, *Staphylococcus aureus*), both yielding a somatic cell count of $\sim 1 \times 10^6$ cells mL^{-1} on curd firmness, as measured by the Optigraph.

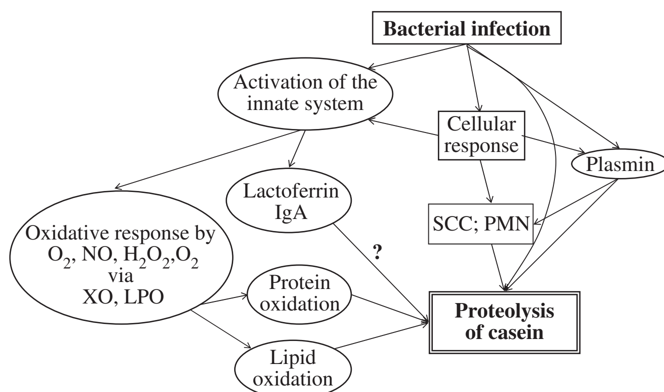

Fig. 2. Schematic representation of effects influencing proteolysis of casein in milk in the udder and during storage before processing. LPO: lactoperoxidase; SCC: somatic cell count; PMN: polymorphonuclear leucocytes; XO: xanthine oxidase.

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References

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on the caseins. *Journal of Dairy Research*, *50*, 45–55.

Auldist, M. J., Coats, S., Sutherland, B. J., Mayes, J. J., & McDowell, G. H. (1996). Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of cheddar cheese. *Journal of Dairy Research*, *63*, 269–280.

Auldist, M. J., & Hubble, I. B. (1998). Effects of mastitis on raw milk and dairy products. *Australian Journal of Dairy Technology*, *53*, 28–36.

Barbano, D. M., Rasmussen, R. R., & Lynch, J. M. (1991). Influence of milk somatic cell count and milk age on cheese yield. *Journal of Dairy Science*, *74*, 369–388.

Concha, C., Holmberg, O., & Astrom, G. (1986). Cells found in non-infected and *Staphylococcus*-infected bovine mammary quarter and their ability to phagocytose fluorescent microspheres. *Journal of Veterinary Medicine Series B—Infectious Diseases and Veterinary Public Health*, *33*, 371–378.

Coulon, J. B., Gasqui, P., Barnouin, J., Ollier, A., Pradel, P., & Pomies, D. (2002). Effect of mastitis and related-germ on milk yield and composition during naturally occurring udder infections in dairy cows. *Animal Research*, *51*, 383–393.

Galina, M. A., Morales, R., Lopez, B., & Carmona, M. A. (1996). Effect of somatic cell count on lactation and soft cheese yield by dairy goats. *Small Ruminant Research*, *2*, 251–257.

Hamman, J., & Kromker, V. (1997). Potential of specific milk composition variables for cow health management. *Livestock Production Science*, *48*, 201–208.

Harmon, R. (1995). Mastitis and milk quality. In F. Harding (Ed.), *Milk quality* (pp. 25–59). London, UK: Blackie Academic and Professional.

Hayes, K. D., Larson, N. K., & Nielsen, S. S. (2005). Activity of the Bacillus polymyxa protease on the bovine plasmin system, in: First IDF *International Symposium on Indigenous Enzymes in Milk (p. 4). University College, Cork, Ireland.*

Heegaard, C. W., Christensen, T., Rasmussen, M. D., Benfeldt, C., Jensen, N. E., Sejrsen, K., et al. (1994). Plasminogen activators in bovine-milk during mastitis, an inflammatory disease. *Fibrinolysis*, *8*, 22–30.

Hogan, S. J., Gonzalez, N. R., Harmon, J. R., Nickerson, C. S., Oliver, P. S., Pankey, J. W., et al. (1999). *Laboratory handbook on bovine mastitis* (revised ed.). Madison, WI, USA: NMC Inc.

Ikonen, T., Morri, S., Tyriseva, A.-M., Ruottinen, O., & Ojala, M. (2003). Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. *Journal of Dairy Science*, *87*, 458–467.

International Dairy Federation. (1985). *Laboratory methods for use in mastitis work. IDF* Document 132, Brussels, Belgium.

Janosi, S., & Baltay, Z. (2004). Correlations among the somatic cell count of individual bulk milk, result of the California Mastitis Test and bacteriological status of the udder in dairy cows. *Acta Veterinaria Hungarica*, *52*, 173–183.

Klei, L., Yun, J., Sapru, A., Lynch, J., Barbano, D. M., Sears, P., et al. (1998). Effects of milk somatic cell count on cottage cheese yield and quality. *Journal of Dairy Science*, *81*, 1205–1213.

Leigh, J. A., & Lincoln, R. A. (1997). *Streptococcus uberis* acquires plasmin activity following growth in the presence of bovine plasminogen through the action of its specific plasminogen activator. *FEMS Microbiology Letters*, *154*, 123–129.

Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., et al. (2004). Changes in milk composition as affected by subclinical mastitis in sheep. *Journal of Dairy Science*, *87*, 46–52.

Leitner, G., Eligulashvily, R., Krifucks, O., Perl, S., & Saran, A. (2003). Immune cell differentiation in mammary gland tissues and milk of cows chronically infected by *Staphylococcus aureus*. *Journal of Veterinary Medicine B*, *50*, 45–52.

Leitner, G., Merin, U., & Silanikove, N. (2004). Changes in milk composition as affected by subclinical mastitis in goats. *Journal of Dairy Science*, *87*, 1719–1726.

Leitner, G., Merin, U., Silanikove, N., Ezra, E., Chaffer, M., Gollop, N., et al. (2004). Effect of subclinical intramammary infection on somatic cell counts, NAGase activity and gross composition of goat's milk. *Journal of Dairy Research*, *71*, 311–315.

Leitner, G., Shoshani, E., Krifucks, O., Chaffer, M., & Saran, A. (2000). Milk leucocyte population patterns in bovine udder infection of different etiology. *Journal of Veterinary Medicine B*, *47*, 581–589.

Le Roux, Y., Colin, O., & Laurent, F. (1995). Proteolysis in samples of quarter milk with varying somatic cell counts: 1. Comparison of some indicators of endogenous proteolysis in milk. *Journal of Dairy Science*, *78*, 1289–1297.

Le Roux, Y., Girardet, J. M., Humbert, G., Laurent, F., & Linden, G. (1995). Proteolysis in samples of quarter milk with varying somatic cell counts: 2. Component PP3 and beta-casein-1P (F29–105 and F29–107) of the proteose-peptone fraction. *Journal of Dairy Science*, *78*, 1298–1305.

Le Roux, Y., Laurent, F., & Moussaoui, F. (2003). Polymorphonuclear proteolytic activity and milk composition change. *Veterinary Research*, *34*, 629–645.

Marshall, R. T. (Ed.). (1992). *Standard methods for the examination of dairy products* (16th ed.). Washington, DC, USA: American Public Health Association.

Moussaoui, F., Michelutti, I., Le Roux, Y., & Laurent, F. (2002). Mechanisms involved in milk endogenous proteolysis induced by a lipopolysaccharide experimental mastitis. *Journal of Dairy Science*, *85*, 2562–2570.

O'Brien, B., Meaney, W. J., McDonagh, D., & Kelly, A. L. (2001). Influence of somatic cell count and storage interval on composition and processing characteristics of milk from cows in late lactation. *Australian Journal of Dairy Technology*, *56*, 213–218.

O'Farrell, I. P., Sheehan, J. J., Wilkinson, M. G., Harrington, D., & Kelly, A. L. (2002). Influence of addition of plasmin or mastitic milk to cheese milk on quality of smear-ripened cheese. *Lait*, *82*, 305–316.

Paape, M. J., Bannerman, D. D., Zhao, X., & Lee, J. W. (2003). The bovine neutrophil: Structure and function in blood and milk. *Veterinary Research*, *34*, 597–627.

Pitkala, A., Haveri, M., Pyorala, S., Myllys, V., & Honkanen-Buzalski, T. (2004). Bovine mastitis in Finland 2001—Prevalence, distribution of bacteria, and antimicrobial resistance. *Journal of Dairy Science*, *87*, 2433–2441.

Politis, I. (1996). Plasminogen activator system: Implication for mammary cell growth and involution. *Journal of Dairy Science*, *79*, 1097–1107.

Politis, I., Bizelis, I., & Rogdakis, E. (2002). The urokinase-plasminogen activator system in ovine macrophages and neutrophils. *Small Ruminant Research*, *44*, 17–23.

Politis, I., Zavizion, B., Cheli, F., & Baldi, A. (2002). Expression of urokinase plasminogen activator receptor in resting and activated bovine neutrophils. *Journal of Dairy Research*, *69*, 195–204.

Rogers, S. A., & Mitchell, G. E. (1994). The relationship between somatic-cell count, composition and manufacturing properties of bulk milk. 6. Cheddar cheese and skim-milk yogurt. *Australian Journal of Dairy Technology*, *49*, 70–74.

SAS Institute. (2000). *JMP® User's guide*. Cary, NC: USA, SAS Institute Inc.

Schaar, J., & Funke, H. (1986). Effect of subclinical mastitis on milk plasminogen and plasmin compared with that on sodium antitrypsin and *N*-acetyl-β-D-glucosaminidase. *Journal of Dairy Research*, *53*, 515–528.

Shamay, A., Leitner, G., Shapiro, F., & Silanikove, N. (2003). Casein hydrolyzates disrupt tight junction integrity and induced involution in cows. *Journal of Dairy Science*, *86*, 1250–1258.

Shamay, A., Mabjeesh, S. J., & Silanikove, N. (2002). Casein-derived phosphopeptides disrupt tight junction integrity, and precipitously dry up milk secretion in goats. *Life Science*, *70*, 2707–2719.

Shamay, A., Shapiro, F., Barash, H., Bruckental, I., & Silanikove, N. (2000). Effect of dexamethasone on milk yield and composition. *Annales de Zootechnie*, *49*, 343–352.

Silanikove, N., Shamay, A., Sinder, D., & Moran, A. (2000). Stress down regulates milk yield in cows by plasmin induced β-casein product that blocks K+ channels on the apical membranes. *Life Science*, *67*, 2201–2212.

Urech, E., Puhan, Z., & Schalibaum, M. S. (1999). Changes in milk protein fraction as affected by subclinical mastitis. *Journal of Dairy Science*, *82*, 2402–2411.

Verdi, R. J., & Barbano, D. M. (1988). Preliminary investigation of the properties of somatic cell proteases. *Journal of Dairy Science*, *71*, 534–538.

Verdi, R. J., Barbano, D. M., Dellavalle, M. E., & Senyk, G. F. (1987). Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milks. *Journal of Dairy Science*, *70*, 230–242.

Ward, P. N., Field, T. R., Rosey, E. L., Abu-Median, A. B., Lincoln, R. A., & Leigh, J. A. (2004). Complex interactions between bovine plasminogen and streptococcal plasminogen activator PauA. *Journal of Molecular Biology*, *342*, 1101–1114.

Wilson, D. J., Gonzalez, R. N., & Das, H. H. (1997). Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects on somatic cell count and milk production. *Journal of Dairy Science*, *80*, 2592–2598.

Zachos, T., Politis, I., Gorewit, R. C., & Barbano, D. M. (1992). Effect of mastitis on plasminogen-activator activity of milk somatic-cells. *Journal of Dairy Research*, *59*, 461–467.

Zavizion, B., White, J. H., & Bramley, A. J. (1997). *Staphylococcus aureus* stimulates urokinase-type plasminogen activator expression by bovine mammary cells. *The Journal of Infectious Diseases*, *176*, 1637–1640.