Factors Affecting Herd Milk Composition and Milk Plasmin at Four Levels of Somatic Cell Counts

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ABSTRACT

A longitudinal study was carried out on samples of bulk tank milk collected from 200 farms for 12 mo to evaluate SCC, plasmin and plasminogen activities, psychrotrophic bacteria count, and protein quality in milk and to examine the proteolytic effects of plasmin on milk proteins. Herds were selected randomly from client lists of two dairies to create four groups based on milk sec of the month before the study; herds were reassigned monthly to one of four groups based on SCC for that month. Overall means were 3.73% fat, 3.13% protein by infrared analysis, 3.16% protein by Kjeldahl analysis, 2.42% casein percentage, 4.65% lactose, 5.43 cells/ml of log SCC, 1.13 U of plasmin, 45.6 U of plasminogen, and log 2.86 cfu/ml of psychrotrophic bacteria. The ANOVA showed a significant effect of month on all factors except SCC, which was fixed by the experimental design. Plasmin and plasminogen activities were high from December to May. Plasmin activities and psychrotroph counts were significantly higher for the high SCC group. Casein percentage and number were significantly higher for the low SCC group. (Key words: milk composition, plasmin, somatic cells, psychrotrophs)

Abbreviation key: PBC = psychrotrophic bacteria count.

INTRODUCTION

Numerous studies have implicated plasmin and its zymogen, plasminogen, in degradation of milk proteins. The reader is directed to the review by Fox and Stepaniak (18). Plasmin-sensitive sites occur in $\alpha_{s1}$-, $\alpha_{s2}$-, and $\beta$-caseins, but $\kappa$-casein is resistant to proteolytic hydrolysis (12, 17). $\gamma$-Caseins are formed by this hydrolysis and are peptide fragments of $\beta$-casein, resulting in reduced casein number (casein as a percentage of total protein) in milk (18). Two additional features of the plasmin system make it particularly detrimental to dairy manufacturing. First, the activity of the enzyme continues indefinitely during cold storage, leading to changes in the coagulation properties of milk (16); second, plasmin activity survives high temperature treatment during processing of dairy products (3, 16, 17, 42). Therefore, elimination or reduction of plasmin from the incoming milk would be desirable. Most previous studies on plasmin have been on milk from limited numbers of individual cows (18), and little information exists on the plasmin and plasminogen contents of bulk tank milk (8, 41). A recent study (35) indicated that treatment of cows with bST reduces the plasmin content of the milk from individual cows, suggesting that the use of bST to increase milk production might have additional desirable effects on the processing properties of milk.

Another factor affecting milk quality is psychrotrophic bacteria, which have optimal survival in low temperatures and can survive the pasteurization process (11, 24, 27, 40). In the dairy industry, psychrotrophs are defined as those bacteria with optimal growth at $\leq 7^\circ C$ (11, 26, 27). Consequently, these bacteria con-
continue to grow during refrigeration of milk. Proteolytic enzymes produced and secreted by these bacteria are responsible for biochemical alterations of the milk and have been associated with a wide number of defects in dairy products, including proteolysis in stored milk, bitter tastes of liquid milk, reduced shelf life, reduced quality and yield of cottage cheese, and increased rennet coagulation time (11, 24, 26, 27). Again, the available data on contents of these organisms in bulk tank milk are limited (6, 11, 40). Available information suggests that tests of bulk tank milk for the contents of plasmin and psychrotrophic bacteria might be helpful in the determination of milk quality. A survey of bulk tank milk was therefore undertaken to evaluate the relationships between the concentrations of plasmin, plasminogen, casein, and casein number in relation to SCC and psychrotrophic bacteria count (PBC).

MATERIALS AND METHODS

Analysis of Fat, Protein, Lactose, and SCC

Two hundred farms associated with two Wisconsin dairies (Alto Dairy Cooperative, Waupun and Grande Cheese Company, Brownsville) were included in a 12-mo study. A total of 2424 samples of bulk tank milk was collected in duplicate. One set of samples was analyzed for fat, protein, lactose, and SCC according to standard methodology (28) by the staff of the individual dairies.

In addition, Grande Cheese Company performed the Kjeldahl analysis (total N x 6.38) on 430 milk samples from both dairies for protein and casein contents (5) to verify discrepancies between the two techniques.

Analysis of PBC

Alto Dairy performed the PBC according to standard methods for examination of dairy products (28). Standard plate counts were performed on the samples. The plates were incubated at 7 ± 1°C for 10 d. Colonies were counted, and the number of psychrotrophic bacteria were reported as PBC per milliliter.

Analysis of Plasmin and Plasminogen

The duplicate set of samples was analyzed at the Dairy Science Laboratory (University of Wisconsin-Madison) for plasmin and plasminogen activities. Collection vials containing .01% merthiolate (vol/vol) to prevent bacterial growth during shipping were sent to the dairies. The samples were stored at 4°C during shipping and were received within 4 to 5 d of collection. Plasmin and plasminogen activities were detected using a modified method of Richardson and Pearce (43). The milk samples were combined with sodium citrate (3:1, vol/vol) and centrifuged (14,000 rpm x 20 min at 22°C) to remove the fat layer. The clear aqueous layer was combined with Tris-merthiolate solution (1:10, vol/vol, for plasmin samples and 1:2, vol/vol, for plasminogen samples). Fifty microliters of 1000 Plough units/ml of urokinase (Sigma Chemical Co., St. Louis, MO), a plasminogen activator, were added to the tubes for plasminogen measurement and incubated at 37°C for 1 h. After incubation, samples were further diluted (1:40 vol/vol) with Tris-merthiolate solution. Fifty microliters of samples and plasmin standard (Sigma Chemical Co.) were dispensed into 96-well, flat-bottom plates (Falcon-Becton Dickinson, Lincoln Park, NJ) in duplicate. One hundred microliters of substrate (Tosyl-Gly-Pro-Lys-4-nitroanilid-acetate, Chromozyme@-PL; Boehringer Mannheim, Indianapolis, IN) were added to each well. Absorbance was read at 405 to 600 nm at 0, 1, 2, 4, and 18 h to determine the kinetics of the enzyme reaction. Plasminogen activity was measured indirectly using urokinase-induced plasmin activity and calculated by difference. One unit of plasmin activity represents the amount of enzyme that produces a change in absorbance of .001 at 405 nm and 37°C when p-nitroanilide is measured.

Cheese Yield Predictions

The following two formulas were used to predict cheese yield: the Van Slyke formula (49), established in 1910, which is based on target composition of cheese, and AOAC formula (13), which provides a crude estimate of yield independent of the type of cheese.

Statistical Analyses

General means, standard deviations, and number of observations were computed for all parameters. Values for SCC and PBC were...
log-transformed to obtain normal distribution for these data. A one-way ANOVA was performed according to the following model:

\[ Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \]

where \( Y_{ij} \) = observation of dependent variable, \( \mu \) = overall mean, \( \alpha_i \) = month (\( i = 1 \ldots 12 \)) in Model 1, SCC group (\( i = 1 \ldots 4 \)) in Model 2, PBC group (\( i = 1 \ldots 4 \)) in Model 3, and \( \epsilon_{ij} \) = residual error.

The Tukey-Kramer (\( P < .05 \)) test determined differences between means. In the data analysis, 12 subclasses for the month effect, 4 subclasses for the effect of SCC group, and 4 subclasses for the effect of PBC were used (Table 1). These statistical analyses were performed with the general linear models procedure of SAS (44) and ANOVA of JMP® (25). The Pearson correlation coefficients were estimated to evaluate linear correlations between all pairs of variables. The cluster analysis (25) was performed to determine the relationship between the variables, analyzed by grouping the variables according to the magnitude and interrelationships based on Pearson correlation coefficients. This multivariate approach used the single linkage (nearest neighbor) method. The results are visualized in a dendogram, a tree diagram of the clusters.

### RESULTS AND DISCUSSION

**Fat, Protein, Lactose, and SCC**

A total of 2424 bulk milk samples was collected; however, because of patron terminations and unforeseen circumstances, the number of samples used for analysis was 2272. Yearly means (±SD) for the analyzed parameters are summarized in Table 2, and the distribution throughout the year is shown in Figure 1. A significant (\( P < .01 \)) effect of the month factor occurred for all parameters except SCC (Table 3). Yearly means for the qualitative parameters ranged within reported values for studies on bulk tank milk (9, 10, 20, 31, 34, 46, 51).

Lactose, fat, protein, and casein contents of milk exhibited changes throughout the year (Figure 1). Variations in these chemical properties of milk were consistent with those found in the literature: lactose in agreement with results of Phelan et al. (34) and fat in agreement with results of Everson (15) and Ng-Kwai-Hang (31).

Previous studies on bulk tank milk evaluated the relationships between SCC and milk proteins (9, 20, 30, 31, 52). Therefore, the qualitative parameters were analyzed according to SCC group classification of the herd to test the effect of SCC on lactose, PBC, and milk protein content (Figure 2). The Kjeldahl values of protein percentage, casein percentage, and casein number according to SCC were 3.20% ± .02, 2.49% ± .26, and 77.6 ± .3, respectively, for group A and 3.13% ± .01, 2.39% ± .01, and 76.4 ± .2, respectively, for group D (Figure 2). Group A herd results were

### Table 1. Categories for each factor used in the statistical analysis.

<table>
<thead>
<tr>
<th>Subclasses</th>
<th>Range of factor</th>
<th>Subclass name</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>Month</td>
<td>1 ... 12</td>
</tr>
<tr>
<td>12</td>
<td>July 1992 to Aug 1993</td>
<td>1 ... 12</td>
</tr>
<tr>
<td>log SCC</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>SCC &lt; 5.0</td>
<td>B</td>
</tr>
<tr>
<td>5.0 ≤ SCC &lt; 5.3</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5.3 ≤ SCC &lt; 5.6</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>SCC ≥ 5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log PBC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PBC &lt; 2.0</td>
<td>A</td>
</tr>
<tr>
<td>2.0 ≤ PBC &lt; 2.7</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>2.7 ≤ PBC &lt; 3.0</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>PBC ≥ 3.0</td>
<td></td>
<td>D</td>
</tr>
</tbody>
</table>

1PBC = Psychrotrophic bacteria count.

### Table 2. Sampling strategy and unadjusted means.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>n</th>
<th>X</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>2272</td>
<td>3.73</td>
<td>.30</td>
</tr>
<tr>
<td>Protein</td>
<td>2272</td>
<td>3.13</td>
<td>.15</td>
</tr>
<tr>
<td>Lactose</td>
<td>2272</td>
<td>4.65</td>
<td>.12</td>
</tr>
<tr>
<td>log SCC</td>
<td>2272</td>
<td>5.43</td>
<td>.29</td>
</tr>
<tr>
<td>log PBC</td>
<td>1116</td>
<td>2.86</td>
<td>.54</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>430</td>
<td>45.6</td>
<td>5.91</td>
</tr>
<tr>
<td>Plasmin</td>
<td>430</td>
<td>1.13</td>
<td>.36</td>
</tr>
<tr>
<td>Protein, %2</td>
<td>430</td>
<td>3.16</td>
<td>.17</td>
</tr>
<tr>
<td>Casein, %2</td>
<td>430</td>
<td>2.42</td>
<td>.15</td>
</tr>
<tr>
<td>Casein number</td>
<td>430</td>
<td>76.8</td>
<td>.02</td>
</tr>
</tbody>
</table>

1PBC = Psychrotrophic bacteria count.

2Random subset of samples.
significantly ($P < .05$) different from all other groups only for casein percentage and casein number. These results are in agreement with previous research (4, 21, 50, 51) that showed that milk with low SCC had higher protein and casein percentages and higher casein number. The reduced protein quantity and quality in combination with increasing SCC led to reductions in predicted cheese yields, confirming results from previous studies on relationships between high SCC milk, such as loss in curd firmness and reduced heat stability during cheese manufacturing (2, 7, 13, 14, 51).

PBC

The yearly mean for 1116 samples analyzed for PBC was 720 cfu/ml (log PBC = 2.86 ± 29). Previous studies (11, 27) have shown that PBC of $5 \times 10^6$ cfu is necessary to detect proteolysis of milk proteins. Adams et al. (1) reported a 10 to 20% decrease in $\kappa$-casein with PBC of $10^5$ cfu. However, different strains of psychrotrophic bacteria produce different proteases with diverse activities (11, 23, 27). A significant ($P < .01$) effect of month occurred for PBC (Table 3). The increase in PBC was different from results of other studies in which PBC was elevated in summer (11, 14, 23, 45, 47). We speculated that the PBC might be elevated in winter because lower temperatures are optimal for proliferation or because inefficient sanitation of milking equipment in the colder season contributes to increased growth of psychrotrophic bacteria (11, 48).

The herds were also ranked by PBC based on quartile distribution. The results of this analysis are shown in Figure 3. A significant ($P < .01$) effect of PBC occurred for fat, protein percentage, and lactose (Table 3). Kjeldahl protein percentage, Kjeldahl casein percentage, and casein number were the highest for PBC group A. The SCC and plasmin activities were significantly ($P < .01$) lower for PBC group A (Figure 3), implying some association between PBC and involvement in the proteolytic degradation of casein fractions.

<p>| TABLE 3. Analysis of variance: effect of month, SCC groups, and PBC1 group on all factors ($r^2$). |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Month</th>
<th>SCC</th>
<th>PBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>.286**</td>
<td>.004</td>
<td>.035**</td>
</tr>
<tr>
<td>Protein</td>
<td>.189**</td>
<td>.003</td>
<td>.016**</td>
</tr>
<tr>
<td>Lactose</td>
<td>.209**</td>
<td>.044**</td>
<td>.13**</td>
</tr>
<tr>
<td>log SCC</td>
<td>.005</td>
<td>.038**</td>
<td>.067**</td>
</tr>
<tr>
<td>Plasmin</td>
<td>.301**</td>
<td>.023**</td>
<td>.067**</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>.533**</td>
<td>.004</td>
<td>.019</td>
</tr>
<tr>
<td>Protein, %</td>
<td>.214**</td>
<td>.012</td>
<td>.026</td>
</tr>
<tr>
<td>Casein, %</td>
<td>.160**</td>
<td>.022*</td>
<td>.020</td>
</tr>
<tr>
<td>Casein number</td>
<td>.028</td>
<td>.020*</td>
<td>.001</td>
</tr>
<tr>
<td>log PBC</td>
<td>.058**</td>
<td>.004</td>
<td></td>
</tr>
</tbody>
</table>

1PBC = Psychrotrophic bacteria count.

* $P < .05$.

** $P < .01$. 

Plasmin and Plasminogen

The yearly means (± SD) for plasmin and plasminogen were 1.13 U ± .36 and 45.6 U ± 5.91 (Table 2). An official method for plasmin and plasminogen detection is not available, and units of activity reported in the literature are numerous, but, when different reported methods were taken into account, our values were within the ranges reported by others (18).

Figure 2. Means of plasmin, log psychrotrophic bacteria count (PBC), Kjeldahl protein percentage, Kjeldahl casein percentage, and casein number for each SCC group.

Importantly, the assayed milk samples were bulk milk and not individual milk samples, which may explain discrepancies in reported measurements of plasmin and plasminogen activities. A significant \( P < .01 \) increase in plasmin and plasminogen occurred from November to May (Figure 1c), confirming the trend reported in previous studies (8, 36, 39, 40).

Figure 3. Means of plasmin, log SCC, Kjeldahl protein percentage, Kjeldahl casein percentage, and casein number for each psychrotrophic bacteria count (PBC) group.

The effect of SCC on plasmin activity was significant \( (P < .01) \), and differences between plasmin activity in groups A and B compared with plasmin activity of group D were .17 and .13 U, respectively, indicating that lower SCC milk may also have reduced plasmin activity. However, the assay used to measure plasmin activity may have included activity measurements of other proteases, further confounding any possible relationships among plasmin, SCC, and PBC.

**Multivariate Analysis**

A multivariate approach was used to obtain more information about the linkages between the parameters analyzed. The correlation analysis, which was used to measure the degree of closeness of linear relationship between the variables, revealed significant relationships between casein percentage, number, and plasmin activity and log SCC \( (r = -.124, -.168, \text{ and } .161) \) and between log PBC and plasmin \( (r = .278) \) and log SCC \( (r = .167) \) but did not clarify any interrelationships between the complexity of variables.

In the experimental design, not all samples were subjected to all analytical procedures; therefore, in the cluster analysis, the complete subset of samples analyzed for all parameters was used. This analysis, based on the Pearson correlation matrix, clusters the variables according to the interrelationships of their correlation coefficients and allows the identification of combinations of factors underlying the biological correlation. The relationships are shown in the dendogram in Figure 4, which permits visualization of the degree of association between the experimental variables. The primary design variable (log SCC) clearly is in a unique cluster associated with the remaining variables. However, associations among the remaining variables are also seen. A close association exists between several indices of manufacturing characteristics of milk: the content of psychrotrophic bacteria and casein percentage and between plasmin activity and casein number. Further, both of these clusters are linked to the content in log SCC, indicating the existence of an interrelationship among PBC, SCC, and plasmin, which contribute to the degradation of the milk caseins.

This approach suggests that, to establish the effect of various factors on milk quality, discriminant analysis enables the identification of complex relationships that a linear analysis does not detect.

**Cheese Yield Predictions**

The Van Slyke formula (49) and the AOAC casein formula (13) were used jointly because divergence between the results may indicate problems in the measurement system and the necessity of monitoring in the cheese-making process. The small discrepancies between the two groups of results may be due to the overestimation of the cheese yield derived from the Van Slyke formula compared with estimation from the AOAC formula, where the N value is 6.25 vs. 6.38 (Figure 5, a and b).

The predicted cheese yield estimated with the AOAC formula and analyzed according to SCC group (Figure 5b) resulted in a difference in cheese yield of .25 kg/100 kg between SCC group A and group D (9.69 kg/100 kg vs. 9.44 kg/100 kg). Although the differences between cheese yields by SCC were not statistically significant, previous studies have shown higher cheese yield for milk with lower SCC (6, 7, 29, 32, 37, 38).

Predicted cheese yield was also analyzed according to PBC group (Figure 5, c and d), and trend was similar to that of the SCC analysis \( (P = .09 \text{ vs. } P = .194) \). The SCC and PBC analyses were not significant, but the same trends were observed for cheese yield. The difference of .3 kg/100 kg between PBC groups A and D may indicate that psychro-
trophic bacteria are responsible for increases in proteolytic activity and casein degradation, thus reducing cheese yield (6, 19). Verdi et al. (52) studied a limited number of psychrotrophic bacteria and found no significant conversion of plasminogen to plasmin, suggesting that the psychrotrophs studied did not produce plasminogen activators and that the conversion occurred through other factors. However, a routine PBC test that detects number and different strains of psychrotrophic bacteria may provide better evaluation of milk quality and cheese yield.

CONCLUSIONS

Milk from this sampling of contemporary herds shows relatively typical changes in composition by month. By design, the evaluation of the association of SCC with other qualitative properties of milk was possible. The analysis showed significant effects of SCC on plasmin activity and association between PBC and SCC. These associations may have important implications on milk quality assessment and should be included in a farm HACCP (Hazard Analysis Critical Control Point) program. Proteolysis is associated with elevated SCC, but there are several contributing factors. Therefore, undue emphasis should not be placed on SCC as the only indicator of milk quality. Psychrotrophic bacteria also affect milk quality in terms of reduced shelf-life and product yield and may be a source of proteolytic enzymes or plasminogen activators.

![Figure 5](image-url)

Figure 5. Predicted cheese yield for the Van Slyke (49) and AOAC formulas (13) by SCC group and by psychrotrophic bacteria count (PBC) group.
At least three potential risk factors have been identified, one of which is SCC. Therefore, a multivariate approach should be used to define quality parameters on which the milk payment schemes might be based. These findings, which are relevant as milk quality standards developed for SCC in cows, are also applied to other milk-producing animals, such as sheep and goats, which appear to have inherently higher SCC in milk (22, 33). Determination of the relationships among SCC, plasmin, and psychrotrophic bacteria may improve quality control systems for milk and dairy products.

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