Evaluation of Milk for Cheese Production Based on Milk Characteristics and Formagraph Measures

R. ALEANDRI, J. C. SCHNEIDER, and L. G. BUTTAZZONI
Associazione Italiana Allevatori
00161 Rome, Italy

ABSTRACT

The characteristics of milk, including its clotting ability, were considered in an effort to develop an equation to predict yield of aged Parmesan cheese. The clotting properties of milk were measured using the Formagraph on 279 milk samples from the vats of 12 cheese plants over 12 mo. A significant, nonlinear relationship was found between the Formagraph measure of curd firmness and cheese yield. The relationship between predicted cheese yield and curd firmness depended on fat level. Increasing curd firmness increased cheese yield only at the low fat level. Intermediate values of curd firmness predicted highest cheese yields for the intermediate fat level. At the high fat level, increasing curd firmness resulted in decreasing predicted cheese yields. Cheese yields increased with fat and protein level. It appears that the Formagraph measure of curd firmness is related to cheese yield as an indicator of casein aggregation properties of the milk. No other Formagraph measures were significantly associated with actual aged cheese yield.

INTRODUCTION

Parmesan cheese is a traditional product of the provinces of Parma and Reggio Emilia in Italy and an important component of the Italian dairy industry, utilizing about 20% of the national milk production annually. It is made from a mixture of whole and partly skimmed milk not less than 1.8% fat. The starter is traditionally the residual serum of the previous day’s cheese production, which is allowed to acidify due to the action of bacteria. Rennet is added anywhere from 10 to 25 min after the addition of the starter and a curd forms about 10 min later. The curd is drained and pressed and the cheese is floated in brine for 20 to 25 d. The cheese may then be aged from 11 to 24 mo. As with any other cheese, the amount of Parmesan cheese produced from a given amount of milk depends on the quantity and the quality of the components of the milk used. The relationships among the milk components constitute a complex system that determines the coagulation properties of the milk. The Formagraph (Foss America, Fishkill, NY) is an instrument developed to measure the clotting properties of a milk sample. Traditionally three measures are associated with the Formagraph, taken from the movement of pendulums immersed in linearly oscillating samples of renneted milk. A light flash at each end of the sample oscillation records the pendulum position on self-developing photographic paper. A normal Formagraph evaluation takes 30 min with the paper moving 2 mm/min. Figure 1 represents a typical graph produced by the Formagraph. The traditional measure of coagulation time, T, is the time (measured in mm) at which the arms of the graph begin to separate. The measure of curd firming rate, K20, is the distance (mm) between the beginning of coagulation and when the arms of the graph achieve a separation of 20 mm. Curd firmness, E30, is the final separation (measured in mm) of the arms at the end of the evaluation.

There has been much interest in the relationship between the composition of milk and the various types of graphs produced by the Formagraph. In general, it is assumed that there is some relationship between the coagulating properties of milk and its capacity to produce cheese. Past evaluations of the Formagraph have focused mainly on its use in evaluating
milk for use in Cheddar (15, 17) or Parmesan cheese production (8, 12). One method by which the Formagraph was studied was to classify cheese making ability of milk samples based on their Formagraph readings and then to analyze the composition of the different classes of milk (18, 23). Other studies have evaluated the relationship of the Formagraph to milk composition and then extrapolated these relationships to cheese yield (6, 16) based on the observation that curd firmness at cutting is a main factor that influences cheese yield (5) and that the Formagraph measurement $E_{30}$ is generally considered as the curd firmness of a particular milk sample.

Significant variations in the Formagraph measures of coagulation time and curd firmness have been found in relation to individual cows (14), addition of calcium chloride (17, 18), chymosin concentration (17, 18), temperature (18), stage of lactation (7, 14), SCC (16), casein composition (10, 15), Soxlet’s acidity (SH) (24), and pH (17, 24). $\kappa$-Casein genotype $BB$, and $\beta$-lactoglobulin genotype $BB$ may have a significant influence (6, 8, 12) as well as the relative amounts of the different caseins (10).

Milk composition was significantly influenced by the stage of lactation (3, 4), SCC (22), and milk protein genotype (13). Based on the relationship of milk characteristics with actual cheese yield and their association with the Formagraph measures, a relationship between the Formagraph evaluation and actual cheese yield has been speculated (14, 23), but the direct relationship between the Formagraph measures and actual cheese yield has not been studied in detail. It is the purpose of this study to determine the relationship of Formagraph readings to actual Parmesan cheese yield and develop a yield prediction equation based on the Formagraph evaluations and other characteristics of the milk.

**MATERIALS AND METHODS**

**Data Collection**

Milk samples were collected monthly from the bell-shaped cooking vats of 12 Parmesan cheese producers over 1 yr beginning in June 1985 and ending in May 1986. All cheese factories were small, traditional operations obtaining milk from a single farm, with no factories receiving milk from the same farm. Samples were collected from the raw vat milk, a mixture of morning milk and skimmed evening milk. The samples were evaluated for four Formagraph (Fosselectric Formagraph type 11710) measures: $T$, $K_{10}$, $K_{20}$, and $E_{30}$. The samples were also evaluated for protein percentage, fat percentage, somatic cells, pH, $^*SH$, and sugar content. The preservative NaN$_3$ was added to the milk samples. Several other components associated with cheese production were also considered: sampling date, amount of milk, amount of acid serum added (an acidified residual from cheese production of the previous day), pH, and $^*SH$ of the milk after acid serum addition, rennet amount, rennet strength, initial temperature, cooking temperature, and cooking time. The total fresh curd yield (after pressing and hooping) and the curd yield after salting (floating in brine for about 20 d) were also recorded. The amount of milk in each vat is known. Normally a vat used for Parmesan cheese contains about 1000 L and produces two
blocks of cheese. Occasionally the producer may use only a half vat due to the availability of milk and produce only one block of cheese. These half lots of cheese tend to be highly irregular, and because of this, it was decided to consider only lots of milk producing 2 blocks of cheese, resulting in 279 usable observations. Total cheese yield was calculated as the sum of the weights of the two blocks produced from one vat of milk after aging for 6 mo.

Statistical Analysis

Based on previous knowledge of cheese making theory, subsets of the independent variables were used in ordinary least squares analysis of covariance in an effort to determine the most significant grouping to predict cheese yield. Initially a model containing all main factors, squares, and interactions was run. The number of variables in the model was reduced by considering their significance level and prior knowledge of the relationships among the variables. In this way several subsets were evaluated and an "optimal" model was chosen based also on the ease of collection of the parameters in the field. The model chosen was:

\[ y_{ijkm} = \mu + P_i + R_j + M_k + b_1x_{1ijkm} + b_2x_{2ijkm} + b_3x_{3ijkm} + b_4x_{4ijkm} + b_5x_{5ijkm} + e_{ijkm} \]

where:

- \( y \) is the 6-mo aged Parmesan cheese yield in kilogram,
- \( \mu \) is the intercept,
- \( P_i \) is the \( i^{th} \) cheese plant \((i = 1,2,3,...,12)\),
- \( R_j \) is the \( j^{th} \) rennet strength group \((j = 1,2,3,4)\),
- \( M_k \) is the \( k^{th} \) sample month \((k = 1,2,3,4,5)\),
- \( x_1 \) is the fat content of milk in kilograms,
- \( x_2 \) is the \( E_{30} \) value of the milk sample,
- \( x_3 \) is the protein content of the milk in kilograms,
- \( x_4 \) is the fat percentage of the milk,
- \( x_5 \) is the grams of rennet added to produce the cheese, and
- \( e \) is the residual error.

The rennet strength class included four numerical levels and a group for missing values. Cheese yield was predicted using only the coefficients for the parameters associated with milk composition (\( E_{30} \), percentage of fat, kilograms of fat, kilograms of protein) to predict the cheese yield and determine the trends in cheese yield with variation in these parameters.

RESULTS AND DISCUSSION

Effect of Classification Factors

The analysis of covariance for cheese yield based on the "optimal", subset of variables is presented in Table 1. The sums of squares reported represent the amount of variability accounted for by that variable after consideration of all others in the model. All variables have highly significant effects \((P>.01)\). Cheese plant accounted for the second greatest proportion of the variability in cheese yield. It was necessary to include cheese plant in the model to adjust for the differences inherent in the processes of each plant, thus accounting for the skill of each cheese maker. The significance of month is in accordance with the reported literature. Okigbo et al. (15) found a highly significant relationship between period of lactation and curd firmness, and Davoli et al. (7) suggested that the Formagraph measures be taken every month due to variability associated with stage of lactation. The measure of rennet strength is essentially a measure of the rennet activity and type.

Effects of Continuous Factors

Although all the measures of the Formagraph were highly correlated to each other, only \( E_{30} \) was significantly associated with cheese yield. Although the overall amount of variability accounted for by the \( E_{30} \) measure is relatively small, it stayed significant when considered with almost any subset of variables. The relationship between \( E_{30} \) and cheese yield was nonlinear. This result is quite different from
TABLE 1. Analysis of covariance of cheese yield.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>232.719</td>
<td>.0001</td>
</tr>
<tr>
<td>Cheese plant</td>
<td>11</td>
<td>451.785</td>
<td>.0001</td>
</tr>
<tr>
<td>Rennet strength</td>
<td>4</td>
<td>63.995</td>
<td>.0013</td>
</tr>
<tr>
<td>Month</td>
<td>11</td>
<td>173.055</td>
<td>.0001</td>
</tr>
<tr>
<td>Fat, kg</td>
<td>1</td>
<td>407.579</td>
<td>.0001</td>
</tr>
<tr>
<td>E30</td>
<td>1</td>
<td>56.189</td>
<td>.0001</td>
</tr>
<tr>
<td>Protein, kg$^2$</td>
<td>1</td>
<td>503.182</td>
<td>.0001</td>
</tr>
<tr>
<td>(E30)$^2$</td>
<td>1</td>
<td>24.430</td>
<td>.0084</td>
</tr>
<tr>
<td>Fat% x E30</td>
<td>1</td>
<td>41.487</td>
<td>.0006</td>
</tr>
<tr>
<td>Rennet, g</td>
<td>1</td>
<td>56.779</td>
<td>.0001</td>
</tr>
<tr>
<td>Error</td>
<td>228</td>
<td>787.798</td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = .933$

that expected from traditional theory, which assumed that there is a direct, linear relationship between E30 and cheese yield. The E30 has been found to be significantly affected by the relative casein composition (10, 15) and casein genotype (6, 8, 12). Marziali and Ng-Kwai-Hang (9) found that higher cheese yields were obtained with genotypes of β-casein BB, κ-casein BB, and β-lactoglobulin genotype BB. Storry and Ford (20) found a curvilinear relationship between total casein concentration and coagulum strength. It would appear that the nonlinear relationships of E30 and amount of protein to cheese yield may be due to the effects of casein concentration and genotype on cheese yield. The quadratic relationship of amount of protein to cheese yield appears plausible, since higher protein percentages appear to be associated with some specific casein genotypes (13) and these genotypes may have some physical properties that improve cheese yield (9). In the preliminary data analysis, a significant relationship was found between certain cheese plants and the protein percentage, but this did little to improve the predictive ability of the model. It is speculated that this interaction was due to the proportions of α₁ casein, β-casein, and κ-casein and lactoglobulin genotypes related to each farm. However, the proportions of genotypes in each vat could not be determined for this study. It appears that E30 is associated with the casein coagulation properties of milk, and its value as a covariate is in adjusting for these differences within cheese plants. Further studies must be conducted to determine the exact nature of the effect of casein on cheese yield.

A significant interaction between E30 and fat kilograms was found that indicates that the response in E30 is not the same for all amounts of fat. Curd firmness and fat were not correlated, which is in agreement with the work of Storry et al. (21). It appears that the amount of fat does not influence the coagulum strength nor is it able to be completely retained during the cheese making process. This result appears to be consistent with the work of Banks (3) who found that the efficiency of fat recovery (retention of fat in the curd) increased with the protein to fat ratio.

The amount of variability accounted for by E30 may have been reduced by its association with the pH of the raw milk. Although the pH of the milk does not influence the Parmesan cheese yield, since the milk is acidified to a pH of about 6.4 by the addition of the starter, it does have some effect on the E30 value. Several studies (14, 16, 24) have found that the curd firmness is negatively influenced by the pH of the milk sample but the persistent significance of E30 in most of our exploratory models indicates that E30 carries more information than the pH of the milk sample alone. Future standardization of milk samples to a pH of 6.4 may improve the value of the E30 measure. The effect of SCC was not significant after the consideration of cheese plant. This is contrary to the results of Okigbo et al. (15, 16), who found significant differences in curd firmness between normal and mastitic milk but also found mastitic milk to be associated with higher pH. It appears that the addition of starter to the milk removes the differences due to SCC.
TABLE 2. Analysis of covariance of flesh curd yield.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>369.475</td>
<td>.0001</td>
</tr>
<tr>
<td>Cheese plant</td>
<td>10</td>
<td>321.542</td>
<td>.0001</td>
</tr>
<tr>
<td>Rennet strength</td>
<td>4</td>
<td>101.032</td>
<td>.0001</td>
</tr>
<tr>
<td>Month</td>
<td>11</td>
<td>141.789</td>
<td>.0001</td>
</tr>
<tr>
<td>Fat, kg</td>
<td>1</td>
<td>439.320</td>
<td>.0001</td>
</tr>
<tr>
<td>E30</td>
<td>1</td>
<td>33.169</td>
<td>.0019</td>
</tr>
<tr>
<td>Protein, kg&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1</td>
<td>451.042</td>
<td>.0001</td>
</tr>
<tr>
<td>(E30)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1</td>
<td>15.963</td>
<td>.0302</td>
</tr>
<tr>
<td>Fat% x E30</td>
<td>1</td>
<td>23.225</td>
<td>.0001</td>
</tr>
<tr>
<td>Rennet, g</td>
<td>1</td>
<td>63.473</td>
<td>.0001</td>
</tr>
<tr>
<td>Error</td>
<td>221</td>
<td>741.446</td>
<td></td>
</tr>
</tbody>
</table>

R<sup>2</sup> = .938

by reducing the pH of the milk. The substantial proteolytic breakdown of α<sub>51</sub>- and β-caseins with elevated SCC found by Verdi et al. (22) either did not occur because cell counts were not as high or do not affect the cheese yield. The amount of rennet was considered because of its importance in the cheese making process (18).

Fit of the Model

The R<sup>2</sup> value of the model predicting 6-mo aged Parmesan cheese yield was .933 (Table 1). The results of the same model run on fresh curd and salted curd are presented in Tables 2 and 3. The R<sup>2</sup> values for the models on fresh curd and salted curd were .938 and .944, respectively. The R<sup>2</sup> of models predicting cheese yield with only technological components were generally around .6 and those with the milk components alone were generally around .8. The aging of the cheese does not appear to have any large effect on the predictive ability of the model. There were, however, some very slight differences in the parameters for the three phases of cheese production.

The coefficients for the milk component covariates from the models on total cheese, total salted curd, and total fresh curd are presented in Table 4. The R<sup>2</sup> of the model predicting cheese yield without any E<sub>30</sub> values was .928. Although the inclusion of E<sub>30</sub> in the model resulted in a very small (.005) increase in the R<sup>2</sup> value of the model, residuals were consistently smaller than those of any model without E<sub>30</sub>. The negative coefficient of interaction E<sub>30</sub> and fat percentage was greatest during the salting stage of cheese production (Ta-

TABLE 3. Analysis of covariance of salted curd yield.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>279.405</td>
<td>.0001</td>
</tr>
<tr>
<td>Cheese plant</td>
<td>10</td>
<td>247.024</td>
<td>.0001</td>
</tr>
<tr>
<td>Rennet strength</td>
<td>4</td>
<td>80.675</td>
<td>.0001</td>
</tr>
<tr>
<td>Month</td>
<td>11</td>
<td>190.964</td>
<td>.0001</td>
</tr>
<tr>
<td>Fat, kg</td>
<td>1</td>
<td>470.314</td>
<td>.0001</td>
</tr>
<tr>
<td>E&lt;sub&gt;30&lt;/sub&gt;</td>
<td>1</td>
<td>51.087</td>
<td>.0001</td>
</tr>
<tr>
<td>Protein, kg&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1</td>
<td>377.452</td>
<td>.0001</td>
</tr>
<tr>
<td>(E&lt;sub&gt;30&lt;/sub&gt;)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1</td>
<td>18.359</td>
<td>.0129</td>
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<tr>
<td>Fat% x E&lt;sub&gt;30&lt;/sub&gt;</td>
<td>1</td>
<td>42.017</td>
<td>.0002</td>
</tr>
<tr>
<td>Rennet, g</td>
<td>1</td>
<td>65.347</td>
<td>.0001</td>
</tr>
<tr>
<td>Error</td>
<td>221</td>
<td>645.440</td>
<td></td>
</tr>
</tbody>
</table>

R<sup>2</sup> = .944
A possible explanation is that curd firmness is a critical factor in the amount of fat that can be retained in the curd. The intercept in this model should theoretically be zero since 0 kg of milk should yield 0 kg of cheese. Thus, it was decided to evaluate the model without the inclusion of the intercept. Only the estimates of the classification factors changed. There was no change in the coefficients of the covariates or in the significance levels of any factors.

**A Prediction Equation**

The prediction equation developed included only the coefficients of the milk characteristic covariates of the model on 6-mo aged cheese plus the intercept (Table 4):

\[
\text{Predicted} = \mu + b_1(fat \text{ kg}) + b_2(E_{30}) + b_3(\text{protein kg}^2) + b_4(E_{30})^2 + b_5(E_{30} \times \text{fat\%})
\]

The prediction equation does not include any factor other than milk characteristics, because the main purpose of the project was to rank milk for its cheese making ability. The inclusion of the intercept term does not affect the ranking of the milk, but it does allow for a more realistic estimate of the potential cheese production. This equation should represent the value of milk for cheese making because all other factors of significance (the cheese factory, month, and rennet type and grams) were considered in the model. Predicted yields were calculated based on this equation. A Pearson product-moment correlation coefficient calculated between these predicted yields and the actual yields was .910. The correlation with the predicted cheese yields from the model without \(E_{30}\) (Table 5) was .906. A formula suggested by Banks (3) was also tested as a comparison. This formula was:

\[
\text{Yield\%} = 1.58(\text{fat\%}) + 1.23(\text{protein\%})
\]

The yield percentage was then multiplied by the amount of milk used to produce the cheese to obtain the predicted yield. The correlation between actual cheese yield and that predicted by this formula was .899. The average difference between the predicted yield and actual yield (as a percentage of the actual yield) was calculated as a measure of accuracy. The equation with \(E_{30}\) underestimated the actual yield by an average of 3.9%, and the equation without \(E_{30}\) underestimated actual yield by an average of 4.2%. Banks’ equation overestimated actual cheese yield by an average of 5.8%. The sum of squares of the difference between predicted and actual cheese yield was smallest for the prediction equation including \(E_{30}\) and greatest for Banks’ equation.

The model was also rerun on a random sample half of the data set to test its fit and ensure that no extreme observations had influenced the original findings. The fit of the model was very similar in terms of the magnitude of the correlation coefficient.
Figure 2. Predicted cheese yield by curd firmness (E30) at 1000 kg milk and 3.16% protein.

Prediction of Cheese Yield

Cheese yields were predicted for a fixed amount of milk (1000 kg) and all possible combination of three values each of protein percentage, fat percentage, and E30, representing the low, mid, and high values found in samples in the data set. Only the coefficients of the covariates for milk composition (and the intercept) were used. Results are presented in Figure 2. The trends are presented within only one protein level because the patterns for cheese yield within fat level were the same at every protein level. Higher protein resulted in higher predicted cheese yield. Three patterns were seen with the changes in E30. Increasing E30 within the low fat level resulted in increasing predicted cheese yields except for the very largest E30 values at which predictions began to decrease slightly. At the intermediate level of fat, predicted cheese yield was highest for the intermediate values of E30. At the highest fat level, increasing E30 resulted in decreasing predicted cheese yield. It should be noted that predictions of cheese yield were consistently greater with increasing fat level. It would appear that a Parmesan cheese made from milk with a large E30 value is limited in its ability to retain fat. Italian Parmesan cheese makers hold...
the belief that the "best" milk (high solids, good ratio of fat to protein) does not always make the best cheese. This appears to be consistent with our results and the work of Banks (3) who found, under experimental conditions, that a higher protein to fat ratio resulted in increased fat recovery in cheese. However, this result could not be confirmed under actual cheese making conditions, possibly due to some other compositional factors. Banks made cheese from the milk of one herd, whereas the cheese plants in his study obtained milk from several sources, introducing the potential for different casein composition to influence the yield. The results of the simulation indicate that the amount and percentage of fat play a significant role in determining the cheese yield and seasonal variations in fat, rather than protein, may have the greatest influence on changes in cheese production over time. Banks also found that there was considerably less variation in the protein percentage by month than there was in the fat percentage. The high predicted cheese yield at the highest fat level with a zero E30 value is difficult to explain. In this case the milk did not coagulate during the 30-min period of the Formagraph evaluation. It could be that in this case the particular milk sample coagulates more slowly and is better able to incorporate and retain the available fat.

The positive quadratic term for kilograms of protein indicates that increasingly greater cheese yields can be obtained with each increase in protein. Assuming 1000 kg of milk, a constant fat percentage and E30 measurement, and 2% protein, the protein can be expected to contribute directly about 6.4 kg of cheese. An increase to 3% will add about 8.4 kg of cheese and a further increase from 3 to 4% will add about 10.8 kg of cheese. Given the assumptions of the literature that most of the casein in the milk is incorporated into the cheese and that milk protein is about 78% casein (3), then it appears that some other components are being trapped by the protein like whey proteins and peptides. Aleandri et al. (1) found that increasing protein percentage was associated with certain casein genotypes and it may be that a higher protein amount not only traps additional milk components but also that these higher protein amounts are associated with casein genotypes, which may improve cheese yield.

CONCLUSIONS

It appears that Formagraph measurement E30 can be valuable in ranking of milk for the production of cheese. Although there may be some concern that the pH of milk samples may effect the relationship of E30 to a cheese such as Parmesan, E30 apparently accounts for some factor beyond pH. Other measures of the Formagraph were of no value in evaluating milk for cheese production. The nonlinear relationship of E30 to cheese yield appears to be plausible according to the literature. The negative interaction between fat percentage and E30 appears to be due to a strong coagulum's inability to retain all the available fat. The value of E30 appears to be as either an indicator of casein composition, casein genotype, or both. An investigation considering the effects of casein composition and casein genotype simultaneously on cheese yield would be helpful in explaining these results.

REFERENCES


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