Characterization of Cheese Curd Ripened with *Penicillium caseicolum* for Producing a Flavor Concentrate

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**ABSTRACT**

Camembert cheese was prepared from homogenized milk containing veal oral lipase and ripened in a loose curd form to speed up the ripening process. Traditional hooped cheese was a control. Free fatty acid titer increased from 58 μmole in the fresh cheese to 359 μmole in control cheese and 2,289 μmole/g of cheese fat in loose curd cheese. After 3 wk of ripening, the cheese had 70% of its total protein as water soluble protein as compared to 39% in the hooped cheese. Polyacrylamide gel electrophoresis showed 86% of α51-caseins and 35% of β-casein had been degraded in the loose curd cheese. Sensory analysis demonstrated that the loose curd process rapidly produced cheese with intense flavor.

**INTRODUCTION**

Mold ripened cheeses have been increasing in popularity, and research has sought to improve their manufacturing methods and shelf-life (21). Particular attention has been paid to the use of Blue and Camembert cheese as an ingredient in processed cheese, salad dressings, and chip dips. Labor requirements and special care in manufacturing and ripening of mold-ripened cheeses have encouraged studies of new procedures for making these cheese varieties.

Much of the research has been related to development of quick-ripened Blue cheese flavor as a substitute for the cheese (6, 16, 21). Homogenization of milk has been used to speed flavor formation during cheese ripening (23). Size of fat globules is reduced and their surface area increased, thus greatly increasing the possibility of fat hydrolysis by lipase (35). According to Kornacki et al. (20), lipolytic preparations of both animal and microbial origin are utilized widely to intensify flavor and to reduce ripening time of cheeses. Other characteristics, such as cheese moisture and salt content or cheese shape, could be changed to obtain shorter ripening time by speeding the breakdown of lipids and protein in cheese (1, 8, 12, 21, 29, 33, 35).

Our work was to evaluate development of Camembert cheese flavor in a loose curd form that could be incorporated directly in other flavored food products. Experiments were under different conditions to acquire information on the influence of cheese shape, salt and moisture content, milk homogenization, and use of a veal oral lipase on pace of the ripening process and final cheese characteristics.

**MATERIALS AND METHODS**

*Preparation of Cheese.* The manufacturing procedure was an adaptation of the method described by Kosikowski (21). Milk at 1.0% fat content was pasteurized at 65°C for 30 min, followed by two-stage homogenization at 126 and 35 kg/cm², at 50°C. With .013% of starter (direct-vat-set) and .026% of rennet (single strength), .006% of veal oral lipase (Lipase Powder No. 600, Miles Laboratories) was added to the milk at 35°C. Coagulation to the proper degree of firmness required about 50 min. After cutting, the cubes were stirred gently for about 20 min, followed by draining the whey. The fresh curd then was dry salted (1.0%) and divided into two parts. One part was dipped into the traditional round molds (11 cm diameter) used for Camembert and the other part...
was placed upon a fine mesh plastic draining mat to be ripened in the form of loose grain curd. Both curds were kept at room temperature (23°C) for 4 h and transferred to a ripening room at 13°C and 95% of relative humidity. Sixteen hours later the curd was sprayed with an aqueous suspension of *Penicillium caseicolum* spores (Cultures C, Laboratoires Roger, France). After 8 days, mold growth could be seen at the cheese surface. Three batches were prepared, and results are averages of two determinations in each test.

Measurements of pH were with a CHEMTRIX Type 60-A digital pH/mV meter equipped with an Orion model 91-63 pH electrode. Fat analysis was by the Roese-Gottlieb method with Mojonnier modification for cheese (26).

Moisture was by the Vacumm Oven Method described by Kosikowski (21). Salt in cheese was assayed by the modified Volhard Test described by Kosikowski (21). Total protein was by the micro-Kjeldahl method, according to the Association of Official Analytical Chemists (4). For soluble protein determination, extraction was according to the method of Sharp (22), followed by nitrogen analysis. Free fatty acids were determined by the rapid silica gel method (15), and volatile acidity was by the rapid direct distillation method of Kosikowski and Dahlberg (22).

Polyacrylamide gel electrophoresis (PAGE) was in 9% polyacrylamide gels in a vertical water-cooled Bio-Rad Model 150-A electrophoresis cell by a method essentially similar to that of Ornstein (28) and Davis (9). Gels were stained for protein with Coomassie Brilliant Blue G 250 (.04%) for 15 h and destained electrolytically in 4% acetic acid for 60 min. Gel rods were scanned at 550 nm with the aid of a Beckman DU spectrophotometer Model 2400 equipped with a gel scanner Model 2520 and a Photometer 252 by Gilford Instrument Laboratories. The system was connected to an HP Integrator Model 3380-S, and relative protein concentration was calculated from integrations on densitograms.

Sensory analysis of the cheese was by a panel of four experienced judges to evaluate flavor in the 12-day ripened samples. A balanced hedonic scale was used to rate samples: 1 point (very mild flavor), 2 points (moderately mild), 3 points (slightly mild), 4 points (neither strong/mild), 5 points (slightly strong), 6 points (moderately strong), to 7 points (very strong flavor). Results were analyzed statistically (analysis of variance) according to the ASTM (3).

**RESULTS AND DISCUSSION**

**Ripening Characteristics**

The composition of fresh Camembert cheese was similar for both loose curd and conventional procedures as the curd was originally from the same batch (Table 1). A high moisture and low salt cheese was obtained to acelerate ripening (9, 10, 11, 12, 35). Jong and Groot-Mostert (19) stated that the most striking differences in physicochemical conditions between cheeses are those of pH, salt, and moisture content. Raadsveld (30) studied protein breakdown in Edam cheese and demonstrated that the soluble N content was highest in the high moisture cheeses studied.

Control and experimental cheeses were ripened for 21 days and then analyzed along with an 86-day ripened commercial Camembert cheese purchased from a supermarket. Results are in Table 1. Considerable differences were observed between characteristics of cheese ripened in the conventional way (hooped cheese) and cheese ripened in a loose form. Having a large surface area, loss of moisture was greater in the loose curd Camembert. However, protein and fat breakdown was much more intense in loose curd than in hooped cheese. Cheese dimensions and format affect the pace of the ripening process (21), and one could speculate that the loose grains allow growth of *P. caseicolum* due to better air circulation and faster migration of enzymes toward the cheese center. Free fatty acid titer (FFA) increased from 58 μmoles/g of cheese fat in the fresh cheese to 359 μmoles in hooped cheese and 2,289 μmoles in the loose curd cheese after 21 days of ripening. The commercial cheese after 86 days of ripening showed 305 μmoles of FFA/g cheese fat. The same trend was observed for protein breakdown. The amount of soluble protein increased from 7.6% in the fresh curd to 39.4 and 70.0% in hooped and loose curd Camembert cheese after 21 days of ripening. Commercial cheese showed 30.8% of its total protein as water soluble protein. Differences may be due to the special treatments.
Table 1. Chemical composition of fresh (1 day) and ripened Camembert cheese (21 days) and commercial Camembert (ripened for 86 days).

<table>
<thead>
<tr>
<th></th>
<th>Fresh cheese</th>
<th>Loose curd cheese</th>
<th>Hooped cheese</th>
<th>Commercial cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>60.1</td>
<td>55.4</td>
<td>58.1</td>
<td>48.1</td>
</tr>
<tr>
<td>Fat in dry matter</td>
<td>34.2</td>
<td>34.2</td>
<td>34.2</td>
<td>46.0</td>
</tr>
<tr>
<td>Volatile fatty acids</td>
<td>12.6</td>
<td>44.8</td>
<td>14.7</td>
<td>18.9</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>57.7</td>
<td>2289.1</td>
<td>359.1</td>
<td>305.2</td>
</tr>
<tr>
<td>Total protein</td>
<td>22.6</td>
<td>26.8</td>
<td>24.4</td>
<td>21.0</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>7.6</td>
<td>70.0</td>
<td>39.4</td>
<td>30.8</td>
</tr>
<tr>
<td>NaCl/moisture</td>
<td>1.0</td>
<td>1.1</td>
<td>1.1</td>
<td>3.6</td>
</tr>
<tr>
<td>pH</td>
<td>5.7</td>
<td>6.6</td>
<td>6.0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

1 All results are the average of duplicate determinations.
2 Expressed as ml N/10 acid per 100 g cheese.
3 μmoles FFA per g of fat in cheese.
4 Expressed as percentage of total protein.
5 Other results, except pH, expressed as percentage.

(milk homogenization, adding of veal oral lipase, and attainment of a cheese with lower salt content) undergone by the loose curd and hooped cheeses during the process. Ripening of loose curd increased proteolysis and lipolysis, compared to hooping the curd in the traditional way. However, the hooped cheese ripened faster than the commercial Camembert cheese, presumably because of its high moisture and low salt content. The higher the moisture in a cheese, the faster the protein breakdown at a given temperature (1, 33). High salt may inhibit protein breakdown in cheeses (1, 8, 30, 33) as well as growth of *P. caseicolum* (14, 31, 32).

In addition, the effect of homogenization, which breaks the fat globules down to a smaller, more uniform size, must be considered (16, 27). According to Wong (35), through homogenization the size of fat globules is reduced and their surface area is increased significantly, thus greatly increasing the possibility of lipolysis. Lane and Hammer (23) found that in Blue cheese made from homogenized milk, hydrolysis of fat was accelerated.

Finally, one must consider the addition of veal oral lipase that brought about an increase in amount of enzymes able to hydrolyze the fat. The production of free fatty acids and volatile fatty acids was much more intense in both curds (loose curd and conventional) compared to commercial Camembert cheese. Fox (11) indicated that treatment of Blue cheese curd with pregastric esterases improves and intensifies its flavor. Other authors found similar results for Blue and other cheeses (2, 5, 7, 17, 20, 35).

**Disc Electrophoresis**

Polyacrylamide gel electrophoresis (PAGE) was used for estimation of α₅₁- and β-casein breakdown during ripening of the cheese. The electrophoretic patterns were compared with electrophoregram of casein freshly prepared from skim milk by HCl precipitation.

Figure 1 shows densitometric patterns of fresh cheese 1) and 21-day ripened loose curd 2) and hooped cheese 3). In densitogram 1 α₅₁-casein is apparent as the leading band, and two peaks of lower electrophoretic mobility can be seen next to this fraction, probably a result of degradation by rennet enzymes. Ledford et al. (24) observed similar results for Cheddar cheese, following overnight pressing. After 21 days of ripening, several degradation products were formed, especially in the cheese ripened in loose form (densitogram 2). The extent of breakdown was considerably less in the cheese ripened in the traditional way, as shown in densitogram 3.
Results of densitometric determinations are summarized in Table 2. The concentration of each protein component was derived from the densitograms. As long as the optical density remained constant, no change of concentration was observed and the protein was considered to be unattacked. Protein loads on the gels were 50 μg for the fresh cheese (1 day) and 200 μg for the ripened cheeses (21 days).

In fresh cheese, αs1-casein represented the larger component and was responsible for an average of 44.4% of all bands, as compared with 37.0% for β-casein. But after 21 days of ripening, the αs1 fraction was reduced to 6.0% and β-casein to 24.2% in the loose curd form. However, in conventionally-ripened cheese, no breakdown was observed in the fraction, as compared to 28.8% of breakdown estimated for αs1-casein. The extent of degradation of αs1-casein in loose curd cheese was 86.5%. Two conclusions can be drawn: first, by ripening the cheese in a loose form, the breakdown of both casein fractions, especially αs1-casein, is appreciably stimulated and, second, β-casein is much more resistant to proteolysis than αs1-casein. The strong attack of rennet and microbial enzymes on αs1-casein has been demonstrated by different authors (13, 18, 25, 29, 34), who found that most of the β-casein remains intact during the ripening of most cheeses.

Organoleptic Assessment

The development of flavor in loose curd Camembert was assessed by organoleptic evaluation of the product at 12 days of ripening as compared to 60-day ripened commercial Camembert cheese. Because the new cheese flavor concentrate is to be used in the manufacture of other food products, it was important to obtain a cheese with flavor comparable to that of a conventional Camembert. The loose curd cheese received an average score of 6.3 (moderately strong flavor) as compared with a score of 4.0 (neither strong nor mild) attributed to the conventional Camembert cheese in the Hedonic scale rating from 1 to 7 points. Statistically, no significant difference was found between these samples (P > .05). As conventional cheese was ripened much longer (more than 60 days) than the loose curd cheese (12 days), it may be inferred that a similar flavor intensity was obtained in a shorter period of time.

<table>
<thead>
<tr>
<th></th>
<th>Fresh cheese</th>
<th>Ripened cheese</th>
<th>Percentage breakdown</th>
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<tbody>
<tr>
<td></td>
<td>β-casein</td>
<td>αs1-casein</td>
<td>β-casein</td>
</tr>
<tr>
<td>Conventional</td>
<td>37.0</td>
<td>44.4</td>
<td>38.9</td>
</tr>
<tr>
<td>Loose curd</td>
<td>37.0</td>
<td>44.4</td>
<td>24.2</td>
</tr>
</tbody>
</table>
Cheese form and thickness affect drastically the pace of the ripening process. The greater area available for the growth of *P. caseicolum* in the loose curd cheese apparently accelerated the ripening process. Both fat hydrolysis and protein breakdown were stimulated in this procedure, bringing about a cheese with intense formation of volatile and total free fatty acids with flavor comparable to that of a conventional Camembert cheese ripened much longer.

Analysis of a commercial Camembert cheese (86-day ripened) demonstrated that homogenization of the milk and addition of a veal oral lipase stimulated the ripening process even if the Camembert cheese were ripened in the traditional wheel format. The process seems to be stimulated also by the presence of low salt and high moisture contents in the cheese.

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