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PROCEEDINGS FROM THE

MARSCHALL ITALIAN & SPECIALTY CHEESE SEMINAR

PRESENTED BY RHÔNE-POULENC DAIRY INGREDIENTS MADISON, WISCONSIN

IN CONJUNCTION WITH SEMINAR EXHIBITORS AND NON-EXHIBITING HOSTS SEPTEMBER 28 & 29, 1994 HOLIDAY INN - MADISON WEST MADISON, WISCONSIN
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INFLUENCE OF CHEESE TEMPERATURE AND SCREW SPEED ON MOZZARELLA CHEESE YIELD

David M. Barbano, Anna Renda¹, J. Joseph Yun, and Paul S. Kindstedt²
Northeast Dairy Foods Research Center
Department of Food Science
Cornell University, Ithaca, NY 14853

¹ Progetto Ibleo, Istituto di Scienze e Tecnologie delle Produzioni Animali, Università di Catania, Italia
² Department of Animal and Food Science
University of Vermont, Burlington 05405

Abstract

The impact of mixer temperature and mixer screw speeds on fat recovery, protein recovery, and yield of Mozzarella cheese was determined. In the first trial three different cheese temperatures were achieved: 130°F (54°C); 140°F (60°C); and 150°F (66°C). In the mixer temperature trial, a screw speed of 12 rpm (revolutions per minute) was used at all mixer temperatures. In the second trial, three different mixer screw speeds were used for stretching: 5 rpm, slow; 12 rpm, medium (ca. 50% of mixer full speed); 19 rpm, fast. Three vats of cheese were made per day. The two trials were conducted at separate times. Cheese making was replicated on three different days in each trial. The important results of the work were the following: 1) increasing screw speed decreased cheese moisture, 2) fat lost in the mixer increased with decreasing mixer temperature and increasing screw speed, 3) no significant influence of mixer temperature or mixer screw speed on nitrogen recovery was detected, 4) cheese yield efficiency increased with increasing stretching temperature and decreasing screw speed due primarily to changes in fat recovery in cheese, and 5) operational conditions in the Mozzarella mixer can have a large influence on cheese yield.

Future work should focus on development of Mozzarella mixer technology that can deliver control of cheese functionality and cheese yield.

Introduction

The pasta filata process of heating and stretching cheese curd is typical of many Italian cheese varieties (7). The traditional process was done by hand combining hot water with curd and kneading the mixture until a proper texture was achieved. This process imparts unique characteristics to the pasta filata type cheeses. Today, single and twin screw mechanical mixers coupled with steam injection systems are typically used for stretching the cheese during manufacture of pasta filata type cheeses (e.g., Mozzarella) in the US. A wide range of operating conditions (temperature, screw speed, feed rate, etc.) are used in industry.

Very little research has been published on the impact of cheese stretching parameters on composition of cheese, losses of fat and protein in stretching water, and cheese yield. Under commercial conditions, the temperatures of stretching water can range from 65 to 85°C. The actual temperature attained in the cheese will be a function of curd feed rate, screw speed, and volume capacity of the mixer. The relationships between mixer operating conditions and final functional characteristics and yield of Mozzarella cheese are not well under-
stood. The objective of the present study was to determine the impact of the temperature of cheese in the mixer and mixer screw speed during stretching on fat recovery, protein recovery and yield of Mozzarella cheese.

MATERIALS AND METHODS

Cheese Making

Low-moisture, part-skim Mozzarella cheese was made on each of three days using a "No-Brine" cheese-making method (5). Raw skim milk and raw cream from the Cornell University dairy plant were combined to obtain a target fat on a dry basis (FDB) in cheese of 38%. The standardized milk was pasteurized at 72°C for 16 s, cooled to 39°F (4°C), divided into three equal portions (about 200 kg each), and stored overnight at 39°F (4°C).

The next day, the milk (about 200 kg per vat) was poured into a cheese vat (model 4MX; Kusel Equipment Co., Watertown, WI) and heated to 38°C. Details of culture, coagulant, and cheese-making conditions up to the point of stretching are as described previously (5).

In the first trial, three different cheese temperatures were achieved: 130°F (54°C); 140°F (60°C); and 150°F (66°C). In the mixer temperature trial, a screw speed of 12 rpm (revolutions per minute) was used at all mixer temperatures. In the second trial, three different mixer screw speeds were used for stretching: 5 rpm, slow; 12 rpm, medium (ca. 50% of mixer full speed); 19 rpm, fast. Three vats of cheese were made per day. The two trials were conducted at separate times. One vat of cheese was made using each mixer temperature or screw speed for each day. The mixer was emptied and cleaned between treatments.

A twin-screw, pilot-scale Mozzarella mixer (model 640; Stainless Steel Fabricating Co., Columbus, WI) containing the circulating salt water (6% salt wt/wt) was used. In the mixer temperature experiment, the temperatures of circulating salt water were 135°F (57°C), 150°F (66°C), and 165°F (74°C) and resulted in the cheese temperatures listed in the paragraph above. A mixer water temperature of 57°C was used to stretch the curd for the screw speed experiment. The temperature of circulating salt water and the mixer jacket were maintained at 57°C during stretching. For both experiments, salted curd was fed into the pilot scale mixer at a rate of about 2 kg/min.

Nine 1.4-kg cylinders of cheese (7.5 cm in diameter x 30 cm long) were made per vat. After 60 minutes of cooling, the cheese was removed from the tube and vacuum packaged in a barrier bag (model B150; Cryovac, Duncan, SC) and stored at 4°C. Cylinder numbers 3 and 4 were used for the analyses of composition.

Milk, Whey, Stretching Water, and Cheese Analyses

Chemical Composition. Changes in titratable acidity of milk and whey (8) and pH of milk, whey, and cheese were monitored during cheese making (9). Fat content of milk, whey and stretching water (1) were determined using Mojonnier ether extraction method; Babcock test was used to measure the fat content in the cream (1) and cheese (8). All nitrogen determinations were made by the Kjeldahl method (1, 3). Percentages of nitrogen from the analyses of noncasein nitrogen (6), nonprotein nitrogen (4) and total nitrogen (1, 3) were multiplied by 6.38 to give milk protein equivalents. All chemical analyses, except for cheese moisture (quadraplicate), cheese fat (quadraplicate) and cheese total nitrogen (triplicate), were performed in duplicate. Cheese moisture was determined gravimetrically, using a forced air oven (model OVEN 490A-2; Blue M, Blue Island, IL) at 100°C for 24 hours (8). Salt content in cheese was deter-
mined by the Volhard procedure (8). Cheese samples for chemical analyses were ground and packed in a 50-ml plastic snap-lid vial, without head space, to minimize moisture loss from cheese during storage at 4°C (up to 2 days prior to analyses).

Yield Measurement

**Fat and Nitrogen Recoveries.** Since fat and protein are the major milk solids in Mozzarella cheese, it is important to account for milk fat and protein distribution in the three products of cheese making, namely whey, stretching water, and cheese. Percent fat recovery in each product was calculated as the weight of each product multiplied by its percent fat content and then divided by the total weight of fat present in the original milk and multiplied by 100. The total fat recovery is the sum of the weight of fat in all products divided by the weight of fat in the milk used for cheese making. Total percent fat recovery will not equal exactly 100%, due to cumulative experimental errors in both fat test and weight measurements. However, total fat accountability should be very near 100%. To neutralize the small differences from vat-to-vat in measured total fat recovery, relative (normalized to 100%) fat recoveries in whey, salt whey, and cheese were calculated.

Total nitrogen recovery (i.e., protein) and relative (or normalized) percentages of nitrogen recovered in whey, stretching water, and cheese were calculated using the same approach.

**Yield Calculations.** Actual cheese yield for each vat of cheese was calculated by dividing the weight of the cheese after cooling by the total weight of milk minus weight of milk samples taken up to the time of rennet addition. Moisture and salt adjusted cheese yield (A|Y) was calculated using an equation (shown below) with a desired cheese moisture of 48.5% and a desired cheese salt content of 1.3%.

\[
\text{A|Y} = \frac{(\text{Actual yield}) [100 - (\text{Actual }\% \text{ moisture} + \% \text{ salt})]}{100 - (\text{Desired }\% \text{ moisture} + \% \text{ salt})}
\]

Theoretical cheese yield (TY) was calculated by the modified Van Slyke cheese yield formula (2).

\[
\text{TY} = \frac{[0.85 \times \% \text{ milk fat}] + (\% \text{ milk casein} - 0.1)] 1.13}{1 - [\text{Desired cheese }\% \text{ moisture} / 100]}
\]

The .85 factor in the formula assumes that 85% of the fat in milk will be retained in the cheese. The equation also assumes that .1% casein will not be recovered in the cheese. The 1.13 factor takes into account a constant factor for retention of added salt and the noncasein and nonfat milk solid components retained in the aqueous phase of the cheese. The desired cheese moisture was 48.5%. Cheese yield efficiency is calculated by dividing the composition adjusted yield by the theoretical cheese yield and multiplying by 100.

**Experimental Design and Statistical Analysis**

Three vats of cheese, each using a different cheese stretching temperature or a different mixer screw speed, were made on one day from one batch of milk. The cheese making was
replicated on three different days. In the mixer temperature trial, a 3 X 3 Latin square design was used. In the screw speed trial, a randomized block design was used. On each day in the screw speed trial, the order of cheese making was randomized for the three different mixer screw speeds. The data for chemical composition and yield were analyzed using PROC ANOVA of SAS (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Mixer Temperature Trial

The fat, protein, and casein contents of milks used in three cheese making days for the mixer temperature trial were (mean ± SD) 2.23 ± .01, 2.23 ± .01, and 2.23 ± .01% for fat, 3.12 ± .1, 3.12 ± .09, and 3.12 ± .08% for protein, and 2.38 ± .07, 2.38 ± .06, and 2.37 ± .06% for casein, for the cheese temperatures of 130°F (54°C); 140°F (60°C), and 150°F (66°C), respectively. Significant differences in stretching water fat content due to mixer temperature were detected: low, .72 ± .10; medium, .22 ± .03; and high .21 ± .01%.

Mixer temperature had a significant effect on cheese moisture and FDB. In general, cheese moisture percentage and FDB increased as mixer temperature increased (Table 1). The increase in FDB with increasing mixer temperature is consistent with significant decrease in fat content of the stretching water with increasing mixer temperature.

Actual total fat recovery in whey, stretching water, and cheese was about 98.25%. When the data were normalized, there was a significantly higher loss of fat in stretching water and a significantly lower recovery of fat in the cheese at the low mixer temperature (Table 2). Actual total nitrogen recovery in whey, stretching water, and cheese was about 100.5%. When the data were normalized, there was a significantly higher loss of protein in stretching water at the low mixer temperature, but the magnitude of the differences in protein losses were much smaller than the differences in fat losses (Table 3).

Differences in cheese temperature in the mixer caused differences in composition adjusted yield and yield efficiency. The composition adjusted yield and yield efficiency were lower at lower mixer temperature (Table 4).

Screw Speed Trial

The fat, protein, and casein contents of milks used in three cheese-making days for the screw speed trial were (mean ± SD) 2.31 ± .03, 2.28 ± .04, and 2.31 ± .03% for fat, 3.21 ± .02, 3.20 ± .02, and 3.21 ± .02% for protein, and 2.48 ± .02, 2.47 ± .01, and 2.48 ± .02% for casein, for the screw speeds of 5, 12, and 19 rpm, respectively. Significant differences in stretching water fat content due to screw speed were detected: slow, .30 ± .05; medium, .75 ± .17; and fast 1.35 ± .17%.

Mixer screw speed had a significant effect on cheese moisture and FDB (Table 5). In general, cheese moisture percentage and FDB decreased as mixer screw speed increased. The decrease in FDB with increasing screw speed is consistent with significant increase in fat content of the stretching water with increasing screw speed.

Actual total fat recovery in whey, stretching water, and cheese was about 99.35%. When the data were normalized, there was a significantly higher loss of fat in stretching water and a significantly lower recovery of fat in the cheese at the fast screw speed (Table 6). Actual total nitrogen recovery in whey, stretching water, and cheese was about 101.7%. When the data were normalized, no significant differences in protein recoveries were observed due to
Differences in screw speed (Table 7).

Differences in mixer screw speed caused differences in composition adjusted yield and yield efficiency that are of economic importance, but they were not significantly different from a statistical viewpoint. The low number of replications of each screw speed makes it difficult to detect significant differences. The composition adjusted yield and yield efficiency were lower at the fast screw speed and this is consistent with the significantly lower recovery of fat in the cheese made with the fast screw speed (Table 8).

Conclusions

1. Cheese moisture decreased with increasing screw speed.

2. Fat lost in the mixer increased with decreasing mixer temperature and increasing screw speed.

3. No significant influence of mixer temperature or mixer screw speed on nitrogen recovery was detected.

4. Cheese yield efficiency increased with increasing stretching temperature and decreasing screw speed due primarily to changes in fat recovery in cheese.

5. Operational conditions in the Mozzarella mixer can have a large influence on cheese yield.

Table 1. Cheese Composition - Stretching Temperature

<table>
<thead>
<tr>
<th>Stretching Temperature</th>
<th>Moisture</th>
<th>Fat on a dry basis</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>54°C</td>
<td>X</td>
<td>46.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>60°C</td>
<td>X</td>
<td>47.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>66°C</td>
<td>X</td>
<td>47.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N = 3 vats for each stretching temperature values with different superscripts are different at P < .05.
### Table 2. Percent Fat Recovery - Stretching Temperature

<table>
<thead>
<tr>
<th>Stretching Temperature</th>
<th>Whey</th>
<th>Stretching Water</th>
<th>Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54°C</td>
<td>13.56</td>
<td>7.52$^a$</td>
<td>78.92$^a$</td>
</tr>
<tr>
<td>60°C</td>
<td>13.81</td>
<td>2.42$^b$</td>
<td>83.78$^b$</td>
</tr>
<tr>
<td>66°C</td>
<td>12.30</td>
<td>2.23$^b$</td>
<td>85.47$^b$</td>
</tr>
</tbody>
</table>

N = 3 vats for each stretching temperature
values with different superscripts are different at $P < .05$.

### Table 3. Percent Nitrogen Recovery - Stretching Temperature

<table>
<thead>
<tr>
<th>Stretching Temperature</th>
<th>Whey</th>
<th>Stretching Water</th>
<th>Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54°C</td>
<td>26.38</td>
<td>.88$^a$</td>
<td>72.74</td>
</tr>
<tr>
<td>60°C</td>
<td>26.23</td>
<td>.53$^b$</td>
<td>73.23</td>
</tr>
<tr>
<td>66°C</td>
<td>26.27</td>
<td>.47$^b$</td>
<td>73.26</td>
</tr>
</tbody>
</table>

N = 3 vats for each stretching temperature
values with different superscripts are different at $P < .05$. 
### Table 4. Cheese Yield

<table>
<thead>
<tr>
<th>Stretching Temperature</th>
<th>N</th>
<th>Composition Adjusted</th>
<th>Theoretical</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kg/100 Kg Milk</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>54°C</td>
<td>X</td>
<td>3</td>
<td>8.72(^a)</td>
<td>9.15(^a)</td>
</tr>
<tr>
<td>60°C</td>
<td>X</td>
<td>3</td>
<td>9.05(^{ab})</td>
<td>9.17(^{ab})</td>
</tr>
<tr>
<td>66°C</td>
<td>X</td>
<td>3</td>
<td>9.10(^b)</td>
<td>9.13(^b)</td>
</tr>
</tbody>
</table>

Values with different superscripts are different at P < .05.

### Table 5. Cheese Composition – Screw Speed

<table>
<thead>
<tr>
<th>Screw Speed (rpm)</th>
<th>Moisture</th>
<th>Fat on a dry basis</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow (5)</td>
<td>X</td>
<td>48.49(^a)</td>
<td>40.14(^a)</td>
</tr>
<tr>
<td>Medium (12)</td>
<td>X</td>
<td>47.81(^{ab})</td>
<td>38.16(^a)</td>
</tr>
<tr>
<td>Fast (19)</td>
<td>X</td>
<td>46.56(^b)</td>
<td>37.48(^b)</td>
</tr>
</tbody>
</table>

N = 3 vats for each screw speed

Values with different superscripts are different at P < .05.
### Table 6. Percent Fat Recovery – Screw Speed

<table>
<thead>
<tr>
<th>Screw Speed (rpm)</th>
<th></th>
<th>Stretching Water</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whey</td>
<td>Water</td>
<td>Cheese</td>
</tr>
<tr>
<td>Slow (5)</td>
<td>$\bar{X}$</td>
<td>12.80</td>
<td>2.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium (12)</td>
<td>$\bar{X}$</td>
<td>12.30</td>
<td>6.61&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fast (19)</td>
<td>$\bar{X}$</td>
<td>13.16</td>
<td>11.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N = 3 vats for each screw speed
values with different superscripts are different at P < .05.

### Table 7. Percent Nitrogen Recovery – Screw Speed

<table>
<thead>
<tr>
<th>Screw Speed (rpm)</th>
<th></th>
<th>Stretching Water</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whey</td>
<td>Water</td>
<td>Cheese</td>
</tr>
<tr>
<td>Slow (5)</td>
<td>$\bar{X}$</td>
<td>25.35</td>
<td>.61</td>
</tr>
<tr>
<td>Medium (12)</td>
<td>$\bar{X}$</td>
<td>24.77</td>
<td>.60</td>
</tr>
<tr>
<td>Fast (19)</td>
<td>$\bar{X}$</td>
<td>25.01</td>
<td>.77</td>
</tr>
</tbody>
</table>

N = 3 vats for each screw speed
values with different superscripts are different at P < .05.
<table>
<thead>
<tr>
<th>Screw Speed</th>
<th>N</th>
<th>Composition</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adjusted Kg/100 Kg Milk</td>
<td>Theoretical Kg/100 Kg Milk</td>
</tr>
<tr>
<td>Slow (5)</td>
<td>3</td>
<td>9.41</td>
<td>9.52</td>
</tr>
<tr>
<td>Medium (12)</td>
<td>3</td>
<td>9.33</td>
<td>9.46</td>
</tr>
<tr>
<td>Fast (19)</td>
<td>3</td>
<td>9.01</td>
<td>9.51</td>
</tr>
</tbody>
</table>

Values with different superscripts differ at P < .05.
REFERENCES


Lipase enzymes in a pure dry state or blended with liquid coagulant (known as rennet paste) enzymes, have been used for years to develop the unique flavor in hard style Italian cheeses. It is these enzymes that have set apart the flavor profiles of Italian cheese from the rest of the world's cheeses.

Today, we would like to address the role and changes taking place in the processing and application of these valuable enzymes.

Historically, prior to World War II, the lipase enzyme was primarily used in combination with the coagulant in a product called "rennet paste." Rennet paste was produced by grinding up the stomach and its contents from young calf, kid goat or lamb. The rennet paste product contained both a milk clotting enzyme and lipase enzyme. Crude as the product was in its origin and application, it provided the Italian cheesemaker a unique flavor development for his Provolone and Romano cheese.

Though the rennet paste played an important role in the development of the ethnic Italian cheese industry, its application did have some drawbacks. Among the drawbacks:

1. Hygiene - Is it proper to use animal tissue/stomach as an ingredient in a finished product like cheese?
2. The inability to effectively standardize the physical and organoleptic characters of the paste to produce a uniform product. Though the cheeses produced with rennet paste developed very high flavor profiles, the consistency of the flavor varied remarkably in intensity and specific characteristics.

Meanwhile, in the United States, the importation of the Italian rennet paste was common until a "stop-action" was given by the United States Department of Agriculture, calling the product impure and not suitable for use as a food ingredient. The domestic Italian cheesemaker, at this time, was faced with trying to duplicate the motherland's "piccante" and "pecorino" type flavors without the use of rennet paste.

In 1946, the discovery of lipolytic enzymes in the pregastric tissues of young ruminates helped eliminate some of the concern of domestic cheesemakers due to the loss of imported paste. It was this discovery that changed the course of lipase enzyme application in the United States. The ability appeared to produce a lipase enzyme(s) that was a free-flowing powder in a standardized strength.

In order to provide our guests full benefit on how far the lipase enzyme technology has advanced, we feel it appropriate to briefly summarize the current manufacturing process used in providing the free-flowing lipase powder (containing the animal tissue).

1. All gullets are received by the processor in a frozen or heavily salted condition.
2. All gullets are uniformly ground.
3. The ground animal tissue is mixed with a carrier such as nonfat dried milk or whey powder.
4. The mixed product is dried by vacuum, freeze-dried or bed process.
5. The dried product is ground to a fine powder.
6. The concentrate would then be standardized and packaged.

**Cheesemaker’s Application**

Lipase enzymes are generally packaged to meet specific vat sizes or milk volumes. The mixing instructions designate the lipase enzymes be mixed with (20 times volume) cool water. After 20-25 minutes being suspended in water with frequent stirs, the slurry is added to the cheese milk after the starter but prior to the coagulant. In the dried powder form the lipase enzyme remains with the animal tissue only to be released when rehydrated in cool water. The water leaches the enzyme out of the animal tissue.

**Consideration**

Though the free flowing powder was an improvement over its predecessors, it brought about other concerns.

When used, such as in cheese processing, the ground gullet bearing the enzyme is added to the vat and blended into the cheese milk. This method suffers from the disadvantages of providing pockets of lipase activity due to incomplete mixing of the solid lipase containing gullet tissue with the liquid cheese milk. The animal tissue would remain with the application and its final destination would be in the cheese or by-products thereof. In the cheese, potential “hot spots” of enzyme activity could result, whereas the animal tissue, not trapped by the cheese, would result in the whey and would interfere with the whey filtration process.

Another concern of the cheese industry is that more customers are feeling the need to produce cheese with physical and organoleptic characteristics that are well designed, constant and will satisfy the consumers needs. As our customers become more conscious of what is needed to produce a consistent final product, so must we as ingredient suppliers.

The above concerns on the use of the free-flowing powder made with animal tissue and the increased demand for lipase enzymes by the food and dairy industries necessitated the development of an improved processing technology for obtaining higher yields from limited supply of animal sources. With this in mind, it has been the objective of Marschall Dairy Ingredients to develop a manufacturing process by which the lipase enzyme could be extracted from the animal tissue as a purified enzyme, dried, standardized and packaged to meet specifications.

**Liquid Lipase - Pilot Trials Initiated**

Liquid lipase Romano cheese trials were run on 2/89 at small scale levels. The samples were split and aged at 43° and 50°F. Both organoleptic and free fatty acid evaluations were conducted at 1, 2, 3 and 7 months.

The liquid kid lipase experimental product was run at three levels against the standard kid lipase powder product in an effort to identify relative activity in flavor development.
The organoleptic evaluation results on the Romano cheese aged at 43° and 50° over seven months have been received. From the analysis, the specific conclusion was that Vat #3 was similar to both flavor intensity and profile to the kid control, Vat #1. Note the LFU's were equivalent for both Vats #1 and #3. The overall conclusion suggests that the kid lipase, which is partially entrapped within the tissue, has activity and accessibility to the substrate equal to the free and readily available liquid extraction.

Romano cheese prepared at 1135 LFU has equivalent flavor intensities and profile for both tissue and extraction lipase. The preliminary analyzed and flavor trial results were positive. The results of the trial would be the seed that would turn the next seven years into an extensive study on the manufacturing process and product performance. Every aspect of the production process and the final lipase product had to be researched; studies on analytical comparisons, free fatty profiles and major scale-up of the process and trial applications.

Today, Marschall Dairy Ingredients has seen favorable results of the liquid lipase extraction system, the improved lipase products it produces and finally the rewards passed on to our customers. Marschall Dairy Ingredients has made the commitment to revamp their existing facility to accommodate the new equipment and initiate the finest patent protected liquid lipase extraction system in the industry.

What does this mean to our customers and interested parties?

1. A completely new non-tissue lipase enzyme in a dried or paste form. Still the same convenience of a powder and paste but one step closer in purity and refinement.

2. A non-tissue enzyme that would completely eliminate any potential “hotspots” due to unreleased enzymes trapped in tissue particles and released in cheese.

3. A non-tissue lipase enzyme that would prevent any risk of plugging the whey filters and the potential loss of whey products due to such action.

4. Quality Italian cheese flavor depends not only on the amount of lipase used but the quality and quantity of each volatile fatty acid that is present. Uniform fatty acid development will be aided by the production of a uniform and stable lipase enzyme product. The lipase extraction system has that potential.

5. As custom manufacturers of pure lipase enzymes, we offer the cheese manufacturer more options in flavor development than any other supplier. Whether the flavor you desire may take on the “Old World” character or the sharpness of the domestic, rennet paste and lipase enzymes can be blended to provide the uniqueness in flavor profiles your company is seeking.
How to Eliminate Bacteriophage and Microbial Contamination in Bulk Starter Tanks

By

Steven Klundt
Soltech, Inc.

Introduction

For years the dairy industry has sought ways to reduce the risk of bacteriophage (phage) contamination within the bulk starter tank. The presence of bacteriophage in a dairy plant is widely recognized, however, keeping phage and other microbial contaminations away from the starter tanks has always been a challenge.

One method for phage elimination within the starter tank, which is gaining greater acceptance, is the use of sterile air membrane filters. Sterile air filters are a natural choice for bulk starter fermentation vessels, since they originally gained their success in critical fermentations. These filters have played a major role in preventing product contamination in fermenters and storage tanks in the biotech, pharmaceutical and electronic industries for the past 15 years. Sterile air filters can perform the same quality filtration for the dairy industry, provided they are applied correctly.

Present Methods For Phage Control

In an effort to eliminate phage from the starter tank, dairies have tried several techniques. No method that I am aware of can provide the absolute assurance of phage elimination that a sterile air membrane filter can.

1) Sealed Fermenters

An air tight fermentation vessel rated for full pressure and vacuum can provide the phage elimination of a sterile air membrane filter. However, sealed fermentation vessels are very rare in the dairy industry and they are very expensive.

2) HEPA Filters

HEPA (high efficient particulate air) filters have been installed in many industries, including dairies, where clean room air is required. These filters originated for clean rooms in the medical, pharmaceutical and electronics facilities. HEPA filters are very efficient heating, ventilating and air conditioning (HVAC) style filters. They are installed in the HVAC duct system to replace less efficient air filters.

Although the expense to install HEPA filters is low to moderate (depending on the existing HVAC filtration system), they are not guaranteed to remove phage and other microbial contamination from the air. The HEPA filters do nothing to eliminate the phage already present in the starter room. In fact, the positive pressure created in the starter room causes the potentially contaminated air to be drawn into the starter tank when the tank is under vacuum.
3) Chlorine Fogging

Many dairy plants will chlorine fog their entire starter room, as often as once per week. Although this method of phage control is relatively inexpensive, it does not guarantee the elimination of phage within the starter tank. Phage can enter the starter room between foggings and be drawn into the starter tank. Chlorine fogging may be an effective method of temporarily eliminating phage, however, it has the disadvantages of being dangerous to workers and causes corrosive damage to equipment and the building.

4) Sterile Air Vent Filter

Sterile air membrane filters have been successfully used for years as tank vent filters. The filters are attached to the only opening on top of the tank allowing only sterile filtered air to enter and exit the tank. This method of sterile air filtration is very effective, providing the filter assembly is pre-sterilized and the tank is otherwise tightly sealed. Using a sterile vent filter is also a relatively inexpensive approach to tank contamination protection. However, tank vent filters rely on sealed tanks, otherwise the air enters through the path of least resistance, ie. tank openings and worn agitator shaft seals. Since most of the starter tanks are not tightly sealed this approach to phage elimination in the starter tank is ineffective.

5) Sterile Air Blanket

As stated earlier, the use of sterile air membrane filters is not new. For non-air tight tanks or when gas sparging is required, sterile air filters under pressure have been used. Sparging gases into liquids in fermenters and beverage carbonators is a common use for sterile air filters. When tanks are not suited for vent filters, ie. starter tanks, they can be blanketed (purged) with sterile air. The positive sterile air pressure within the tank prevents unwanted contaminants from entering the tank. Through the proper use of sterile air membrane filters, this method has proven totally effective at eliminating phage and other contaminents from entering a vessel. This is a relatively inexpensive approach to phage elimination in the starter tank once the equipment is purchased and installed.

Filtration Principles

What are these sterile air membrane filters that are so highly recommended for the elimination of bacteriophage in starter tanks? These specialized air filters are generally made in cylindrical cartridge form and were introduced in the late 1970’s when filter membrane materials such as PTFE (Teflon®), PVDF (Kynar®), Nylon, and Polysulfone were discovered. Not all sterile membrane filters are recommended for air nor have they all been tested for bacteriophage removal.

There are three primary mechanisms of particulate filtration (figure 1). These mechanisms pertain to both liquid and air filtration. “Direct Interception” is the process of removing particles that are larger than the filter's pore size (sieve filtration). “Inertial Impaction” occurs when a particle lodges within the filters media, becoming trapped by the tortuous path through the media and is unable to be carried out by the fluid flow. “Diffusional Interception” involves the physics of Brownian Motion and van der Waals Forces. Very small particles rapidly ricochet off the filter media’s pores, ultimately being held within the media by weak van der Waals Forces.
It is the last two filtration mechanisms mentioned above that apply primarily to air filters. Very small contaminants, like bacteriophage, are removed by filters with removal efficiencies greater than the contaminant, due to these mechanisms. A proper sterile grade filter will have both a liquid and gas removal rating. The gas removal rating will always be better than the liquid rating.

Figure 1. Mechanisms of filtration

Prefilters

Whenever steam is in direct contact with a food product or is used to sterilize food processing equipment, the steam should be adequately filtered. General plant utility steam will almost always contain scale and other contaminants from the boiler and steam piping. These steam contaminants should be removed prior to the steam entering the mechanical equipment, sterile air filter or the vessel. A good steam filter is simply a high temperature, high efficiency particulate filter, usually made of stainless steel.

A high efficiency coalescing filter is the proper choice as a prefilter to the sterile air filter when using plant compressed air. Coalescing filters are designed to remove oil and water aerosols along with particulate contamination which is present in most compressed air systems. An ordinary particulate prefilter will not remove oil and water which can cause plugging of the sterile air filter. Coalescing filters work on the principle of combining (coalescing) small aerosol droplets into larger and larger droplets (figure 2). The large droplets eventually drop from the filter, by gravity, into the bottom of the filter housing. The collected liquid is periodically drained from the filter housing.
Sterile Air Filters

Because many sterile air filter applications are exposed to moist environments, it is essential that the air filter has a sterile rating for both liquid and air. When filtering compressed air, liquid aerosols and water vapor are often present. Should the filter become wetted, microbial contamination could pass through the filter with the liquid aerosol or droplets. Therefore, the sterile air filter must have a sterile liquid rating. Along with a sterile liquid and gas rating, the filter should be naturally hydrophobic (liquid repelling). Hydrophobic filters made of PTFE and PVDF will not become saturated, experiencing high pressure drops like nonhydrophobic filters.

Sterile filters, like sterile products, have various connotations within different industries. Typically, commercial sterility is the absence of viable pathogenic microorganisms and the reduction of the number of spoilage microorganisms to a level below which the product is stable under normal storage conditions. For example, sterile filtration of beer could mean the removal of yeasts, molds and the microorganism pediococcus, all of which will cause beer to spoil if held at ambient temperatures for a period of time. However, the Food and Drug Administration (FDA) has a much more definitive definition of a sterile filter for the pharmaceutical industry.

The FDA requires a sterile filter to meet a minimum Titre Reduction of $10^6$ organisms/unit volume/cm² area. Titre Reduction ($T_R$) is simply the ratio of influent organisms to effluent organisms during a filter challenge test. For instance, 100,000,000 organisms/unit volume influent to the filter with an effluent of 10 organisms yields a $T_R$ of $10^5$.

$$T_R = \frac{\text{Influent organisms}}{\text{Effluent Organisms}} \quad \text{example from above} \quad T_R = 10^6 = 10^5$$

The contaminant used for the above filter challenge is the microorganism Pseudomonas Diminuta which has a relative size of 0.3 x 0.8 microns. Any liquid filter having a Titre Reduction of $10^6$ using this microorganism can be classified as a 0.2 micron sterile grade liquid filter. However, because bacteriophage can be as small as 20 nanometers (0.02 microns), not all sterile grade liquid filters will remove 100% of the bacteriophage (figure 3).
For elimination of bacteriophage, it is necessary to use a filter that has been challenged successfully against bacteriophage.

**Figure 3. Bacteriophage size and shape**

<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces Bacteriophage G4</td>
<td>30 nm</td>
</tr>
<tr>
<td>Bacteriophage MS-2</td>
<td>20 nm</td>
</tr>
<tr>
<td>Bacteriophage T7</td>
<td>60 nm</td>
</tr>
<tr>
<td>Bacteriophage T1</td>
<td>100 nm</td>
</tr>
</tbody>
</table>

Other important features of a good sterile air filter are capability of being in-line steam sterilized, able to withstand multiple steam sterilization cycles, and integrity testable.

Examples of industries and equipment presently using sterile air filters include manufacturers of aseptic packaging equipment, fruit juice and fluid milk filling machines, fermentation facilities with tank vents and air/gas sparging, the beer and soda industries for carbonation sparging of CO2 gas, pharmaceutical and food industries for transferring products via pressurized air within the piping system.

**Air & Steam Quality**

To eliminate bacteriophage from the starter tank using a sterile compressed air blanket, a suitable sterile air filter is required. At present the 3-A accepted practices for supplying air under pressure in contact with milk, milk products and product contact surfaces (Number 604-03) requires only compressor air intake filters, coalescing filters, and filtration efficiency of 50% by DOP* test.

*DOP = Dioctylphthalate fog method (MIL-Std-282), equal to a typical 15 micron filter.

Steam that is in contact with a food product should be of culinary grade. The present 3-A accepted practices for producing steam of culinary quality (number 609-00) are potable water, acceptable boiler chemical additives (CFR Title 21), and filtration to remove all particles 5 micron and larger.

**Sterilizing Methods**

Sterilizing procedures presently available to industry include thermal - steam or dry heat, filtration, ETO (Ethylene oxide gas), Gamma irradiation, Ultra violet light (UV), and chemicals. Because of logistics and safety considerations, ETO and Gamma irradiation are generally not practical for most production facilities. These two methods are typically used to
pre-sterilize sealed equipment, raw ingredients and finished drugs for the medical and labora-
tory markets. Thermal and chemical sterilization would not be practical for maintaining sterility of the starter tank head space. UV sterilization has been tried within the head space of vessels as well as in liquid and gas streams. This method has proven to be marginally successful and non-absolute primarily because the UV light is susceptible to fouling from contaminants in the liquid and gas streams.

Thermal sterilization of the pressurized air is not economical, because the air must be continually heated and cooled prior to entering the starter vessel. Therefore, the only practical method is to sterile filter the air into the starter vessel and steam sterilize the filter and related components.

Sterile Air System

A sterile air filter along with other separate components can be assembled and piped to each starter tank at the plant. However, it has been shown that a pre-packaged sterile air system offers a more compact, easier to operate, and often less costly system. Packaged sterile air systems have been designed for manual or automated operation with up to a five tank capacity per system. The manual systems require an operator to open and close a series of valves. Automated systems have been designed to have all functions performed automatically with the initiation of a start switch.

Features to look for in a packaged sterile air system include dual filtered compressed air, filtered steam, regulated air and steam pressure, instrumentation, insulated steam lines, and safety pressure and vacuum relief vents.

Conclusion

Sterile air membrane filtration has been around for many years and has proven to be successful in eliminating contamination in fermenters. Some industries and many companies continue to do things the old way and in many cases they get by. It is not uncommon to still find fermentation facilities using canisters packed with steel wool fiber as their air filter. This technology is 25 years old, but they survive, sometimes due to luck but usually because their product is hearty and less susceptible to contamination. Eventually these companies will lose product which could have been saved or they will find their competitors yielding a better product because the competitor made advances to help improve product quality. An affordable sterile air blanketing system can be designed for any starter tank which will eliminate bacteriophage and microbial contamination from entering the vessel. There is no need to lose bulk starter due to bacteriophage.
THE CHANGING ROLE OF CHEESE

By

Donna Gorski, Technical Editor, Dairy Foods Magazine

1. Overview of Cheese Market

When the Food Pyramid and NLEA were introduced to the U.S. population, the cheese industry expressed great concern about how consumers would react to learning that cheese, a food previously recognized as being nutrient-dense and an excellent source of calcium, was also predominantly fat. Cheese began to face a public relations problem primarily because of its fat content. However, it seems that consumers ignore the fat content of cheese when it's used as an ingredient in a prepared food, i.e., entree, pizza, salad, etc. Concerns about health apparently are no worry with people who want food that's more flavorful and easy to prepare.

Of the three primary markets for cheese products—foodservice or restaurants, ingredients in other products, and retail grocery stores—sales to the first two markets have been growing the fastest. Thus, most of the cheese produced in America is not sold to consumers. Dining out, frozen dinners, sandwiches and pizza account for the greatest usage.

Graph #1

CHEESE SHARE OF VOLUME – 1992
By Channels of Distribution
(6.6 billion lbs. cheese, excluding cottage cheese)

FOODSERVICE
40.0%
(2.65 billion lbs.)

INGREDIENT
25.0%
(1.65 billion lbs.)

RETAIL
35.0%
(2.3 billion lbs.)

SOURCE: Business Trend Analysts Inc.
According to Business Trend Analysts, Commack, N.Y., over 6.6 billion lbs. of cheese (excluding cottage cheese) were utilized in the United States in 1992. Of that total, about 1.65 billion lbs. (25%) were sold to other food manufacturers to be used as an ingredient. Roughly 35%, or 2.3 billion lbs., was sold at retail to be consumed at home. Over 2.6 billion lbs. were sold to foodservice outlets for away-from-home consumption, making this the largest single market for cheese, comprising about 40%.

Graph #2

U.S. NATURAL CHEESE PER CAPITA CONSUMPTION
1992

Because of the increase in cheese consumption at restaurants and as an ingredient, per capita consumption of cheese has risen nearly 60% over the past 15 years. That's 26 lbs. of cheese per year per person.
Graph #3

NATURAL CHEESE PER CAPITA CONSUMPTION

YEAR

POUNDS

SOURCE: National Cheese Institute
FOODSERVICE INDUSTRY SALES

$ BILLION

0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300

70 75 80 85 90 93

YEAR

SOURCE: NATIONAL RESTAURANT ASSOCIATION, OCT. 92
American-style cheeses are losing share to other types of cheeses, mainly Italian. In the early 1980s, American cheese accounted for over half of total U.S. cheese production. By 1992, American's share of total production had fallen to 40%. Italian cheeses are driving the industry.

Graph #5

PRODUCTION OF NATURAL CHEESE

Billion lbs.

Italian cheese and specialty cheese are gaining reputations from chefs and culinary schools as "magic ingredients." A specialty cheese can be defined as "a unique cheese of high-quality that may also have unusual characteristics giving it a higher value than typical 'commodity' cheese."

Whereas cheese was often considered a commodity product, with many consumers looking to cheese as an economical alternative source of protein, consumers are learning to appreciate cheese as a delicacy.

Consumers are willing to pay extra for hand-grated Romano at an Italian Bistro, for freshly crumbled feta on a Greek salad, or for rich, mascarpone-filled tiramisu with a cup of espresso.

This changing role of cheese presents an opportunity for small cheesemakers to abandon their traditional American-style production and move to the specialty cheese arena.
Competing with commodity cheese giants won’t get the small guy anywhere, but supplying high-quality, ingredient cheese to the generation of consumers that fear high-fat foods but savor trendy gourmet dishes will most likely increase sales. These cheese are perceived as full of value, not full of fat.

2. Marketing Cheese

Cheese is one of the most versatile food products. It appeals to varied palates and is suitable for almost all age groups. It is consumed at breakfast, lunch, dinner and as a snack. It can be served alone; in dips, sauces and soups; as a complement to sandwiches or entrees; as a seasoning for vegetables and side dishes; as a topping in salads and breads; and in desserts.

Because of the fat fear, smart marketers are repositioning cheese as a natural partner to the grain and vegetable category. Flavorful cheeses are being used in reduced amounts to ‘spike-up’ prepared foods without contributing significant calories. This isn’t to say that reduced-fat cheese isn’t a big market.

The Wisconsin Milk Marketing Board (WMMB) has been very active in this area. Earlier this year they sponsored a Chef Bistro contest where six chefs from around the country prepared three dishes: appetizer, entree and dessert, all featuring Wisconsin cheese. The purpose of the contest was to promote Wisconsin cheese as an ingredient cheese.

Additional efforts include a promotion piece with Cooking Light magazine. The October issue will highlight the use of Wisconsin specialty cheeses in several dishes developed by five of the nation’s hottest chefs. It’s part of the kick-off of WMMB’s new national retail promotion that is built around the dietary concepts of balance and moderation, demonstrating how cheese fits into today’s active lifestyles.

These efforts resulted from concerns about the new nutritional labels. The point: cheese is a dietary ally, not enemy. It has a strong nutritional profile and has flavors to enhance other foods.

Retail programs sponsored by WMMB include a Wisconsin cheese recipe guide filled with cheese and food pairing ideas and nutrition facts, free with purchase. They’ve also published a brochure entitled: Wisconsin Cheese, the Perfect Ingredient for Today’s Prepared Foods. The brochure states that a 1993 analysis of new product data collected between 1990 and 1993 found that cheese was used as an ingredient in 35% of new meals and entrees, 22% of sauces, 15% of salad dressings, and 13% of rice dishes.
LOWFAT AND REGULAR CHEESE CONSUMPTION
1993 SHARE OF RETAIL VOLUME

REDUCED FAT
13%

REGULAR
87%

SOURCE: National Dairy Promotion and Research Board
One must not forget reduced-fat cheese. The light or nutritional cheese segment, with $400-500 million in sales, is expected to grow 20-30% annually over the next several years, according to Business Trend Analysts. The nutritional cheese market continues to be driven by a more health-conscious consumer, concerns over cholesterol and an aging population. Low-fat cheese maintains approximately 13% share of retail volume.

The industry is striving to put low-fat cheeses in the dairy case, but some admit the product now available has yet to meet consumer expectations. Whether or not low-fat cheese eventually becomes as common as lowfat milk, as some predict, the introduction of a slew of new 'healthy' reduced-fat products is expected to lead to a costly shakeout in that sector of the cheese market, according to Business Trend Analysts.

Successful marketing appeals to consumer's needs and delivers. This has been difficult in the reduced-fat cheese arena. When marketing your reduced-fat cheese as an ingredient, suggest using a small amount of highly flavored, full-fat cheese with the reduced-fat version to assist with delivering texture and taste.

The key to marketing full-fat cheese as an ingredient is to select highly flavored varieties, reducing the required usage level. Co-branding can add value, i.e. "made with Maytag Blue."

III. What's New in Cheese
* Innovative cheeses
  Adding peppercorns, unique shredded blends
* Specialty cheeses
  Mascarpone, feta, Gorgonzola
* Healthy cheeses
  Use of fat replacers, technologies and procedural modifications.
  - proteins, carbohydrates, hydrocolloids, milkfat fractions, reduced cholesterol
  - nutritional/reduced-fat mozzarella for school lunch program

IV. Cheese for the 21st Century
The opportunities are endless. Be creative.
BIOFILMS IN CHEESE PROCESSING ENVIRONMENTS

Amy C. Lee Wong
Associate Professor
Food Research Institute
Department of Food Microbiology and Toxicology
University of Wisconsin-Madison

Introduction

Microbial adhesion to surfaces and the development of biofilms are known to occur in many environments. Biofilms consist of microorganisms attached to a substratum (surface), and are frequently embedded in a polymeric matrix produced by the microorganisms. Other organic and inorganic substances and particulate matter from the environment also may be entrapped in the biofilm matrix.

Biofilms can be beneficial in certain natural environments and engineered biological systems. Examples include biofilms in natural streams and fluidized beds used for wastewater treatment which can degrade or trap toxic materials. On the other hand, biofilms are undesirable in many instances and create economic and health problems. For example, biofilm accumulation on ship hulls, water pipes, oil rigs, and heat exchangers causes decreased performance; on implanted medical devices such as catheters and prostheses can lead to chronic infections; and in food processing environments may cause product spoilage or transmission of diseases.

Biofilm development

Biofilm development is a dynamic multistep process (1). When a surface is exposed to an environment, it becomes coated with organic molecules from the liquid phase forming a ‘conditioning’ film. In dairy operations, this film may contain milk proteins and fat molecules. Bacterial cells are transported to the surface and attach. Some of these cells become irreversibly attached and, under suitable conditions, grow and develop into biofilms. The biofilm cells excrete extracellular polymeric substances (EPS), forming a matrix (often referred to as glyocalyx), which helps to stabilize the biofilm. The composition of EPS is generally not well defined; however, polysaccharides are presumed to be a major component. The biofilm is generally heterogeneous in microbial composition and does not necessarily exist as a uniform layer throughout the attachment surface. Other organic and inorganic components from the fluid phase can deposit, thus increasing biofilm accumulation. Subsequently, portions of the biofilm may detach and are removed or redeposit on other surfaces.

Bacterial attachment, EPS production, biofilm formation and detachment are affected by a combination of factors; these include the organisms present, attachment surface, nutrient availability, and physical factors such as temperature, pH, and degree of turbulence. Cells in biofilms are generally harder than their planktonic (free-living) counterparts and exhibit increased resistance to antibiotics, disinfectants, and heat (2, 5). It is speculated that the EPS plays a role in conferring resistance (8), but the mechanisms have not been defined.
Biofilms in dairy environments

Biofilm accumulation in any food environment is a concern. The surfaces of equipment used for food handling, storage or processing are recognized as major sources of microbial contamination. It has been shown that even with acceptable cleaned-in-place (CIP) systems in milking and dairy environments, microorganisms can remain on equipment surfaces (4, 10, 11, 16). These organisms may survive for prolonged periods depending on the amount and nature of residual soil, temperature, and relative humidity (12).

Rubber gaskets are prone to biofilm development. Czechowski (3) found that the number of bacteria recovered from gaskets increased with the length of time the gaskets were in use. Dead ends, joints and valves are also more susceptible to biofilm accumulation. In addition, equipment surfaces can be corroded with age. Pits and cracks may develop where soil and bacteria can collect.

Attachment and biofilm formation by *Listeria monocytogenes* on stainless steel and rubber.

Surveys indicated that *Listeria spp.* could be isolated from milk processing and dairy plants (13). The highest incidence was associated with wet locations such as conveyor belts and drains.

*Listeria monocytogenes* is a foodborne pathogen of significant concern to the dairy industry. We investigated the attachment and biofilm formation by *L. monocytogenes* on stainless steel (SS) and buna-n (BN), a rubber compound commonly used in gaskets. Our results indicate that both processes involve multifaceted interactions between the bacterial cell, attachment surface, and the surrounding microenvironment. Milk and milk proteins (casein, alpha-lactalbumin, and beta-lactoglobulin) significantly reduced attachment (6) by the organism. Surfaces presoiled with these components had a similar effect. An interesting finding was that buna-n inhibited growth of *L. monocytogenes* and several other foodborne pathogens. Other rubber formulations were tested, but none was nearly as inhibitory as buna-n.

Milk soil may be present on equipment surfaces, especially in hard to clean areas such as gaskets and joints. We examined the interactions of residual soil, surface, temperature (6C and 25C) and relative humidity (32.5% and 75.5% RH) on survival of *L. monocytogenes* (7). In the absence of milk soil, survival was highest at 6C and 75.5% RH, and organisms could be recovered from both the SS and BN surfaces after 10 d. With less favorable temperature/RH combinations, the bacteria still survived for 3-5 d. The presence of milk soil provided harbor and nutrient for attached *L. monocytogenes*. The organisms grew on SS at 25C and 75.5% RH. While growth was not observed on BN, the organisms remained close to initial levels for at least 10 d. *L. monocytogenes* persisted in the presence of raw milk natural flora, and was recovered from both SS and BN surfaces under all storage conditions after 10 d. In addition, at 25C and 75.5% RH, the organisms grew on SS despite high numbers of competing raw milk flora.

The nutrient environment and attachment surface can also affect biofilm formation by *L. monocytogenes* and its resistance to sanitizers (15). Under low nutrient conditions, biofilm formation on BN was inhibited for 4 of 7 strains tested. With a high nutrient medium, the effect of BN was not as significant, but the degree of biofilm formation was still lower on BN than on SS. We examined the effectiveness of four types of commonly used sanitizers on biofilm bacteria. Biofilm *L. monocytogenes* cells were much more resistant than their planktonic counterparts, a finding consistent with results reported by other researchers (5, 9, 10). We
also found that biofilm bacteria on BN were much more resistant to the action of sanitizers than those on SS.

**Biofilm formation by nonstarter lactic acid bacteria**

Nonstarter lactic acid bacteria (NSLAB) are common causes of cheese defects such as undesirable flavors, gas formation, or white haze from calcium lactate crystals. Gas formation can cause texture defects and unsightly swollen packages. Calcium lactate is harmless, but consumers often mistake the white haze for mold growth and reject the cheese. These defects pose an economic burden to cheesemakers and dairy farmers. It is estimated that in Wisconsin, the annual loss resulting from calcium lactate haze alone is several million dollars.

The source of these NSLAB is believed to be primarily from post-pasteurization contamination through contact with air or equipment (14). We initiated a series of studies to examine the potential for biofilm formation and contamination of cheese by NSLAB. SS chips (1 in²) were placed at different locations on the inside of a cheese vat and removed at various intervals during cheese-making, cleaning and sanitizing. A calcium lactate crystal-forming *Lactobacillus casei* was added to milk at the same time as the starter culture. The *L. casei* survived in low numbers through the salting step of Cheddar cheese making and also after the vat had been manually cleaned and sanitized. After sanitizing, a second vat was made without the addition of the *L. casei* culture. Cross-contamination from the first vat occurred as evidenced by the recovery of *L. casei* from cheese made in the second vat. Calcium lactate crystals were observed to develop on the surface of the cheese during ripening. Studies are also being conducted to test the potential for biofilms of *L. casei* formed on SS to detach during cheese-making and contaminate the cheese. Preliminary results indicate that biofilm bacteria can be a source of contamination.

**Summary**

Biofilm formation is not a new phenomenon. However, it has received attention in the food arena only recently. Biofilms can serve as a source of bacterial contamination leading to product spoilage or transmission of disease. Bacteria in biofilms are physiologically different from their free-living counterparts. A major concern is their apparent increased resistance to adverse conditions such as exposure to heat and disinfectants. Additional research is needed to fully understand the interactions between the biofilm bacteria, attachment surface, and cleaning agents, so that effective strategies for prevention of biofilm formation and biofilm removal can be implemented.

**References:**


IMAGE ANALYSIS METHOD TO MEASURE BLISTER SIZE AND DISTRIBUTION ON PIZZA

J. Joseph Yun and David M. Barbano
Northeast Dairy Foods Research Center
Cornell University, Ithaca, NY

Ernest F. Bond and Milos Kalab
Centre for Food and Animal Research
Agriculture Canada, Ottawa, Ont.

Abstract

Blisters on pizza, produced during the baking, is an important “after-cook” quality attribute. However, no published information is available on a quantitative method of evaluating blister size and distribution on pizza. In this paper, we described the image analysis program developed to objectively evaluate blisters. The program can provide information on count, area, and color intensity of blisters on pizza. The program is also flexible enough to handle various pizza size and subsections within a pizza. The distance between blisters and the shape of blisters can also be determined. Using the image analysis program, blisters on pizza affected by the age of Mozzarella cheese were compared. During the 8 weeks of storage at 4°C, the number of blisters on a pizza decreased, area per blisters increased slightly, the total area covered by blisters decreased, and the blister color turned slightly lighter. This image analysis program can be used as a quantitative and objective method to measure size and distribution of blisters on pizza.

Introduction

The increase in popularity of pizza has led to the growth of the Mozzarella cheese industry. In 1992, the per capita annual consumption of Mozzarella cheese in the U.S.A. was 7.7 lb (National Cheese Institute, 1993). In 1993, the annual sales of the pizza industry was about $30 billion, and the total value of cheese sold for pizza is estimated to be over $3 billion in the U.S.A. (Anon., 1994). A significant portion of the sales are through pizza chains like Pizza Hut (Anon., 1994). These pizza chains impose strict specifications on Mozzarella cheese quality to the cheese supplier. Although the specifications may be different depending on the pizza chains, the important criteria for “after-cook” quality generally include flavor, color, stretch, and melt as well as size, coverage, and color of blister (Alvarez, 1986).

Blisters on pizza are produced during the baking. Upon heating, the cheese melts, and then air bubbles (formed within the cheese or below the cheese) pushes the melted cheese upward. Then the lifted cheese surface is further subjected to the heat, which changes the color of the surface to brown through Maillard reaction. The intensity of browning is affected by baking conditions, i.e., time and temperature of the oven, as well as the concentrations of reactive amino acid and carbohydrate in the cheese (Johnson and Olson, 1985; Ames, 1992; Hutkins, 1993).

There may be a number of factors affecting the size, coverage, and color of blister. The fact that 3 out of 7 “after-cook” criteria deal with blister characteristics indicates the importance of blister on overall functional quality of Mozzarella cheese in pizza (Alvarez, 1986).
Currently, there is no published information on quantitative method of evaluating blister size and distribution on pizza. In this paper, we described the image analysis program developed for the purpose of quantitatively measuring and objectively evaluating blisters. Using the image analysis program, the count, area coverage, size, and color intensity of blisters on pizza affected by the age of Mozzarella cheese were compared.

**MATERIALS AND METHODS**

**Cheese Making and Chemical Analysis**

Low-moisture, part-skim Mozzarella cheese was produced using the no-brine method as described before (Barbano et al., 1994, Yun et al., 1994). Chemical composition of fresh cheese was determined as described previously (Yun et al., 1993). To determine the effect of storage, the Mozzarella cheese was stored up to 8 weeks at 4°C before the baking test. Pizza baking and image analysis were done in quadruplicate. Age-related chemical changes in Mozzarella cheese, i.e., soluble nitrogen and intact casein contents were also monitored by Kjeldahl and electrophoretic methods (Yun et al., 1993).

**Pizza Baking and Photography**

Frozen pizza crusts (12" in diameter) were purchased (SP Pizza Crust, Canandaigua, NY) and stored overnight at refrigeration temperature for thawing. Pizza sauce (150 g) was spread evenly over the surface leaving 1" on the edge without sauce. Mozzarella cheese was shredded using an electric slicer/shredder (model Professional SaladShooter, National Presto Industries, Eau Claire, WI), and the shredded cheese (300 g) was sprinkled over the sauce on pizza crust. The pizza was baked without adding any toppings at 450°F for 5 min using a conveyor oven (model Impinger II, Lincoln Food Service Products Inc., Ft. Wayne, IN).

After cooling the pizza at room temperature for 30 min, the pizza was placed under 4 photo flood lights (250W each). Photographs were taken using slide film (Ektachrome model 64T, Kodak, Rochester, NY) and a 35 mm camera (model T70, Canon, Tokyo, Japan) with shutter speed of 1/15 of a second and f-stop of F8. The slides (frame size 2" x 2") were developed in a local photoshop.

**Image Analysis**

The slides of pizza pictures were evaluated using a digital image analyzer (model IBAS, Kontron Electronic GmbH, Munich, Germany). The image analysis program was written using IBM compatible software (Bond et al., 1994). The program contained the following subunits: parameter definition, image input, image processing, spatial scaling, background filtering, measuring and filing, and graphic output.

In the parameter definition mode, the measurement parameters such as blister count, blister area, and grey value were specified. The program can also determine blister shape and the distance from one blister to another. In addition, all the information on pizza blister can be determined after subsectioning the pizza picture into 3 equal areas.

The pizza image was digitized at 512 x 512 pixel spatial resolution. Minimum and maximum grey scale values were defined to compensate for the overall lighting conditions. The image was then corrected for uneven illumination of the pizza during photography. The total
of 256 shades of grey scale was used. The higher the number, the lighter the blister color was.

For spatial scaling, a ruler (12") was photographed with each pizza to serve as reference. The area in the pizza where no blisters were present was identified and separated from the blisters. Then the grey scale of the background was separately measured before measuring the information on blisters. The data were filed and composite images including the input and processed images were created and printed as well as the histogram and quantitative information on each parameter.

RESULTS AND DISCUSSIONS

Overall Performance of the Program

The image analysis program provides reproducible information on count, area, and color intensity of blisters on pizza. The program is also flexible enough to handle various pizza size, and evaluation of subsections within a pizza is possible, too. Other criteria such as the distance between blisters and the shape of blisters can also be determined, if desired. There are a number of steps from pizza baking to image analysis that can cause variations in the results. For example, the exact placement of shredded cheese onto pizza can differ slightly within a pizza and also from one pizza to another although the total amount of cheese may be the same. These sources of variations would have to be controlled to make the program more repeatable.

Effect of Storage

The composition of milk used in making Mozzarella cheese for the storage experiment was 2.20% fat, 3.13% total nitrogen, and 2.41% casein nitrogen. Whey composition was 0.30% fat and 0.90% total nitrogen, and the stretching water composition was 0.57% fat and 0.14% total nitrogen. The chemical composition of the fresh cheese was 46.3% moisture, 21.5% fat, 40.0% fat on a dry basis, 27.0% total nitrogen, and 1.77% salt.

As shown in Table 1, the number of blisters on pizza decreased from 291 to 190, from week 2 to week 8, respectively. During the same period, the area for individual blister increased slightly (from 28.5 mm² to 31.3), but the total area covered by blisters decreased from 16.5% of the pizza to 12.0%. The storage caused the blister color to be slightly lighter (98 in grey scale to 115).

During the 8 weeks of storage at 4°C, proteolytic changes in the cheese continued by the action of residual coagulant and starter culture enzymes. Therefore, the soluble nitrogen content of the cheese increased (Figure 1), and intact α-casein content decreased (Figure 2) during the period. The changes in the proteolytic status affect many functional characteristics including the blister formation. Extensive proteolysis makes the cheese texture softer, causing the cheese to melt more easily upon baking and individual blister size to be larger. The lighter color of blisters for the aged cheese may be related to the reduced amount of reactive carbohydrates available as the cheese ages or the increase in free oil release that coats the surface of the blister and reduces drying at the blister surface.
Conclusions

The image analysis program described in this paper can provide an objective means of evaluating blisters on pizza. The size, area coverage, and color intensity of the blisters can be determined quantitatively. This program can serve as a useful tool to study the relationship between manufacturing/storage conditions and functionality of Mozzarella cheese. Future development in making the image analysis program more user-friendly and lower-cost would lead to wider availability of a quantitative method to measure blisters on pizza.

References


Figure 1.
Changes in soluble nitrogen contents, as a percent of total nitrogen, in Mozzarella cheese during 8 weeks of storage at 4°C.

**Figure: Soluble Nitrogen in Mozzarella Cheese**

*Effect of Storage*

- pH 4.6 Sol. N.
- 12% TCA Sol. N.
Figure 2.
Changes in alpha-casein content, as a percent of total protein, in Mozzarella cheese during 8 weeks of storage at 4°C.

Alpha-casein in Mozzarella Cheese
(Effect of Storage)

Table 1.
Count, total area, individual area, and grey level of blisters on pizza made with Mozzarella cheese stored up to 8 weeks at 4°C.

<table>
<thead>
<tr>
<th></th>
<th>2 week old</th>
<th>4 week old</th>
<th>8 week old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>291.3 ± 18.5</td>
<td>238.3 ± 25.8</td>
<td>190.3 ± 2.1</td>
</tr>
<tr>
<td>Total Area, % of Pizza</td>
<td>16.5 ± 1.1</td>
<td>16.1 ± 3.1</td>
<td>12.0 ± 0.4</td>
</tr>
<tr>
<td>Individual Area, mm²</td>
<td>28.5 ± 3.7</td>
<td>33.8 ± 4.9</td>
<td>31.3 ± 1.1</td>
</tr>
<tr>
<td>Grey Level</td>
<td>98.9 ± 4.5</td>
<td>96.6 ± 5.9</td>
<td>115.0 ± 9.1</td>
</tr>
</tbody>
</table>
Good Afternoon Ladies and Gentlemen

This afternoon we'll focus on the manufacture of Mexican cheeses, specifically fresh cheeses or high moisture content cheeses.

There are several varieties of Mexican cheeses including Chihuahua (similar to Cheddar), Manchego and the biggest percentage, fresh cheeses; Queso Crema and Queso Doble Crema, Queso Sierra, Queso Fresco o de Morral, and Queso Oaxaca or Asadero. Originally these cheeses had been made on farms, but with time things have changed and they are now made in plants.

Mexican cheese plants today pasteurize and have some equipment with new technology. And cheesemakers are becoming more involved and looking for better ways of making cheeses in place of relying on old techniques. Smaller factories are disappearing; medium factories are disappearing or growing, getting larger to become stronger and meet new challenges.

We will look at three cheeses considered the most popular in Mexico after Oaxaca and Asadero which are as popular as Mozzarella in the U. S. The main difference is that they are braided or rolled up in a very long string.

The three cheeses we will look at are Panela and Ranchero, both high in moisture, and Cotija, of medium moisture.

PANELA

Milk: Milk is pasteurized and can be added to rennet as whole milk as some years ago or as partially skimmed, standardizing to 2.6% fat. Acidity should be around Q.13 - 0.18°/o

Additives: Calcium chloride lQ-40 Gr/100 Lts. (1-1.5 oz/26.42 gal.)

Setting: Temperature can be set at 30-40°C (86-104°F). For a really soft cheese with high moisture content and good cut characteristics, use lower temperatures.

Cutting: Cut the curd in big grains (1 sq. in.), agitate very slowt for 10-15 minutes and drain approximately half of the whey, continuing to agitate.

Salting: Salt can be added directly or diluted in water first, approximately 1 Kg./100 liters (2.2 lb./26.42 gal.), then agitate for 5-8 minutes.

Molding: Use typical baskets for molding. Then refrigerate and sell.
Ranchero (cheese from the ranch)

Standardization must be adjusted to 3.9% of fat. Pasteurization is at high temperature for a short time.

Setting: A starter is used in about 0.25-0.5% of mesophillic cultures and kept at 35°C (95°F). Formerly cultures were not added to this cheese because milk was not pasteurized. Add rennet, calcium chloride, same as panela. Coagulate and cut after 20-30 minutes. After cutting, wait for five minutes and stir slowly to avoid breaking curd cubes. Heat up to 39°C (102.2°F) for 30 minutes.

After vat wheying off, place the curd in cloth bags and hang them at refrigeration temperature overnight to improve syneresis and so that it reaches desired acidity.

Milling: When the curd is totally wheyed off, mill all the curd in a mill such as used for meat in hamburgers. Following milling, add salt to the mass and mix (2.2 lbs./26.42 gal.)

The molding is an important stage for this cheese. The mass is formed, by hands into a metal ring. The cheese can then be stored for 24-48 hours at refrigeration temperatures.

COTIJA

Milk is received in the standardization tanks with agitation. Fat is 29.9%. Add calcium chloride (2.2 lbs./26.42 gal.) and keep temperatures at 35-36°C (95-97°F). Formerly Cotija was made from raw milk, but now is pasteurized, batch or HTST.

Homogenization:

120 Kg./cm² is the pressure of homogenization at 70°C (158°F), then is backed off to balance the tank. To set, add mesophillic and some thermophillic cultures. Next the milk goes from the balance tank to vats where rennet is added when the temperature reaches 37°C (98.6°F) and wait for 90 minutes until curd has been formed and pH reaches five (5).

Next start cutting with 3/8” knives. Eliminate almost all whey from the vat. Add 6.38 kg./1000 lts. (2.89 lbs./264 gal.) of salt by hand or mixer and mix well. Molding is complete by 24 hours.

Ripening and packing:

4 days - 15-20°C (59-68°F), then 3 days - 6-8°C (41-45°F). Return to the ripening room at 18°C (64.4°F). Store at 6 - 8°C (41 - 46.4°F) and the cheese is ready to sell.
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