7-29-1982

Progress Report for the Dairy Research Advisory Board

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July 12, 1983

Gary H. Richardson

Dear Dr. Richardson:

I have enclosed a copy of the Agenda for the Dairy Research Advisory Board meeting scheduled for July 28, 1983. The meeting will begin at 8:30 a.m. in room 202 of the Nutrition and Food Science Building on the Utah State University campus. The building is located near the North-East corner of 1200 East and 700 North in Logan. At 11:50 a.m. buses will take everyone to the Taggart Student Center for lunch in the West Colony Room. Buses will return to the meeting room at 1:00 p.m. where the project report meeting will continue. We should be finished by about 4:00 p.m.

We appreciate your support of this program and look forward to seeing you on July 28.

Written copies of all reports will be provided to members of the Advisory Board.

Sincerely yours,

C. A. Ernestrom
Dept. Head

lm
Dairy Research Advisory Board
Utah State University
Nutrition and Food Sciences - Room 202
July 28, 1983

AGENDA

8:30 a.m. Welcome - Dr. Doyle J. Matthews, Director, Utah State University Agriculture Experiment Station

8:40 a.m. Project Review

1. Process cheese model systems - C. A. Ernstrom

2. Control of melting properties in process cheese made from U.F. whole milk retentate - Shereif Anis

3. Cottage cheese from ultrafiltered skim milk - Ron Rayner


5. Demonstration of rapid farm test for antibiotics in milk - CEM Corporation

Lunch West Colony Room - Taggart Student Center

1:00 p.m. 6. Rapid direct test for casein in milk - Bob Carpenter

7. Assay for residual pepsin in cheese curd - Gehreyath Majeed

8. Use of proteinase negative cultures in Cheddar cheese making - Lauris Davis

9. Selection of lactic cultures - the basis of proteolytic activity - G. H. Richardson

10. Effect of heat treatment on functional properties of dried whey - Ron Malouf
The first single pair culture program in the USA was introduced into a cheese plant 15 months ago. Now close to 60 cheese plants are using this culture without rotation. They are using the latest transfer technology involving a disposable syringe-needle and rubber septum system. We feel the best "back up" cultures to be protease negative (Prt-) cultures and that they could ultimately replace the single pair because of superior properties.

The Prt- lactic streptococci have been successfully used to manufacture and increase the yield of Cheddar cheese. Studies are currently underway to evaluate Prt- cultures in the manufacture of cottage cheese, buttermilk, sour cream and in reducing the make time for Cheddar cheese. There should be considerable advantages for these strains in yield and in body improvement in these fermented dairy foods. We have isolated 19 Prt- strains and evaluated their performance in milk. There are in these isolates different levels of protease activity as indicated by the different inoculum levels required to develop desired target pH values in milk. All are significantly less proteolytic than Prt+ strains but they do vary in rate of proteolysis. These differences could produce different yield and flavor responses and therefore methodology is needed to evaluate residual protease activity and correlate it with effects upon product manufacture.
We have looked at the growth and acid production of these strains in milk. The presence of 5% Prt+ cells could be detected in Prt- cultures. The Prt+ cultures slowed up when the amount of Prt- variants exceeded about 50%. Interactions between strains is being evaluated using membrane filters and the Spiral Plater. Recently, better protease activity tests have been reported (Church et al. J. Dairy Sci. 66:1219, 1983) which we would like to use in further characterizing Prt- cultures. The recently invented "Vatimer" will be used to evaluate the quality of Prt- coagula. Highly proteolytic cultures should reduce the $G_{\infty}$ values associated with milk coagulation curves. Efforts to isolate Prt- variants of the lactic cultures used in Italian cheese manufacture have not been successful. We would like to continue isolation and identification of the plasmid patterns of the mesophilic and thermophilic cultures. Such studies can augment the growth in milk data to assure that the best Prt- strain is chosen for dairy product manufacture.
Good Text for Yield Est.

GinF = Casein = soluble
Effective Casein = effective

GinF

Rest gran; caseins with
Phenol extraction; McMurry
Citrate + phenole; Scott
Lactic cultures; re-treatment
Bacterial Choice + San Away

Grain - 1973 - 2400 from 76
PROTEASE NEGATIVE CULTURES - CHEDDAR CHEESE MANUFACTURE
Craig Oberg and Lauris Davis

Background

A distinction has been made among strains of *Streptococcus lactis* and *Streptococcus cremoris* concerning protease activity. Those designated protease positive can coagulate milk at a 1% inoculum level in less than 24 hours when incubated at 22°C. Protease negative strains take much longer since they can only use previously available amino acids and peptides for their nitrogen. Negative cultures have not been used for cheesemaking for several reasons including tradition, higher vat inoculum levels needed, longer makes times, and more expensive starter media. Protease negative cultures do offer potential advantages including phage resistance in the cheese vat, decreased protein loss, fewer bitter flavors during curing, and more control over acid production.

Purpose and Methods

The purpose of this research is to examine the feasibility of making Cheddar cheese from protease negative cultures. This study includes different media and conditions of incubation for starter cultures, inocula levels, and acid production. Cheese was also made from protease positive starter cultures grown in pH control media and Marstar MSM media. These were used as the control cheeses and for comparison in the aging study. Yield, nitrogen content of the whey, protein content of the cheese, and differences in aging will be included. This study should show if Cheddar cheese can be made economically with protease negative cultures and illustrate their advantages.

Nine trial vats containing 640 pounds of milk have been made from each starter type. The cheese was cut into five pound blocks and aged.
at 45°F. Analysis is being run on the milk, whey, and cheese. The milk was tested for fat and protein content with a Multispec infrared milk analyzer and total solids with the AOAC method. Cheese and milk fat was also determined using the Babcock fat test. Total nitrogen is being determined by AOAC Kjeldahl method.

Aging analysis on each vat of protease negative and protease positive cheese will be performed for one year to determine any differences between the two cultures. Aging and protein breakdown of the cheese samples is being followed by three methods. Expert cheese graders will grade the cheese at 15, 60, 90, 180, and 365 days of age. Soluble nitrogen as a percent of the total nitrogen will be determined using the Vakalens and Price method at 30, 60, 90, 180, and 365 days. Polyacrylamide urea gel electrophoresis comparisons of protein banding patterns are being run at 30, 60, 90, 180, and 365 days of age.

**Starters**

In order to produce sufficient amounts of starter culture special experimental tanks and an incubation reservoir were constructed. Seven liter stainless steel tanks were fitted with lids containing an inoculation port, a pH electrode port, and an electric motor driven mixer. The incubation reservoir held four such tanks and a water heater. One protease positive media consisted of 5% whey solids and 1.25% Biolac media, with the other containing 11.6% MSM. The protease negative media contained 0.4% yeast extract, 0.1% casein hydrolyzate, and 5% whey solids. The starter media was heat treated by flowing steam in an autoclave. The treatment was comparable to that recommended and done in commercial plants. The tanks were incubated at 24°C in the water bath.

Cultures used included Chris Hansen's 253 and *Streptococcus cremoris* UC73 (-) (a protease negative). Protease activity was determined
by the coagulation time of each culture in 10% NFDM at 30°C. The Hansen's
253 coagulated in eight hours while the S. cremoris UC73 (-) took 24 hours.
One ml of the Hansens 253 frozen concentrate was used for six liters of
protease positive media while 15 ml of coagulated NFDM was used for 6.5
liters of protease negative cultures. All cultures were ready after 18
hours although the negative cultures could be used after 13-15 hours.

In the cheese manufacture activity tests gave rough estimates
for the inocula levels of each starter into the vat. The pH controlled
whey base starter was used at a 0.7% level and the MSM at 1.4%. After
several trials it was found that 2.0% of the protease negative starters
gave good make times.

Results

The cheese manufacture was completed in June with analysis now taking
place. All the cheese produced had acceptable moisture levels and good
body and texture. Acid production during each make was consistent, with
make times averaging 4.5 hours. Statistical analysis of total solids
cheese yield with our methods indicated no difference between starters.
We feel the total nitrogen studies using the Kjeldahl method, particularly
of the whey samples, will give a better indication of any protein loss
due to the starter organisms. These are now being run.

Although most of the cheese is still young (60-90 days), grading
has shown that all starters are comparable with the cheese being of
excellent quality. Cheese flavor development seems to be progressing
well in both the positive and negative cheese. Polyacrylamide gels
show a breakdown of the \( \alpha \)-casein in cheese samples but no difference
between starters. Results to this point show that excellent quality
cheddar cheese can be made from protease negative starter cultures with
reasonable amounts of starter and make times.
Protease negative (Prt-) lactic streptococci have been successfully used to manufacture and increase the yield of Cheddar cheese. Studies are currently underway to evaluate Prt-cultures in the manufacture of cottage cheese, buttermilk, sour cream and in reducing the make time for Cheddar cheese. There should be considerable advantages for these strains in yield and in body improvement in these fermented dairy foods. We have isolated 19 Prt- strains and evaluated their performance in milk. There are in these isolates different levels of "negativity" or protease activity. All are significantly less proteolytic than Prt+ strains but they do vary in rate of proteolysis. These differences could produce different yield and flavor responses and therefore methodology is needed to evaluate residual protease activity and correlate it with effects upon product manufacture.

We have looked at the growth and acid production of these strains in milk. Recently, better protease activity tests have been reported (Church et al. J. Dairy Sci. 66: 1983) which we would like to use in further characterizing Prt- cultures. We would also like to continue isolation and identification of the plasmid patterns of these cultures. Such studies can augment the growth in milk data to assure that the best Prt- cells are chosen for dairy product manufacture.
June 18, 1982

Mr. Fritz Leeman
Brewster Dairies, Inc.
675 South Wabash
Brewster, OH 44613

Dear Mr. Leeman:

We have scheduled the next meeting of the Dairy Research Advisory Board for July 29, 1982. This year's meeting will be held in rooms 307 and 309, new Eccles Conference Center on campus at Utah State University.

The meeting is scheduled to begin at 8:30 AM. Lunch will be in the Taggart Student Center at 12:00 noon.

Progress reports will be given on the research projects approved at the last meeting. Proposals will be made for next year's research activities, and a budget for 1982-83 will be presented. The meeting should be completed by 3:30 PM.

We look forward to your attendance. Please feel free to bring other members of your organization if you would like to.

Sincerely yours,

C.A. Ernstrom
Department Head
DAIRY RESEARCH ADVISORY BOARD
Utah State University
Eccles Conference Center - Room 307-309
July 29, 1982

AGENDA

8:30 AM  Welcome: Dr. Doyle J. Matthews, Director, Utah Agricultural Experiment Station

8:40 AM  Project Review:

1. Growth of *Phodopseudomonas sphaeroides* in whey and whey permeate. Robert Reinbold

2. Selection and testing of single strain cultures for cheese factories. Aly Gamay

3. Use of proteinase negative cultures in cheese manufacture. G.H. Richardson

4. Production of cheese curd from ultrafiltered whole milk. Paul Savello

Lunch

5. Developments in end product pricing of milk for dairy plants. C.A. Ernstrom and R.J. Brown

6. A rapid direct test for casein in milk. Robert Carpenter

7. Variations in the coagulation properties of milk from individual cows. Leslie Okigbo

8. Microbiological changes during production of curd from ultrafiltered whole milk. Gordon Brown
SUGGESTED PROJECTS FOR 1982-83

1. Use of *Rhodo pseudomonas* for the fermentation of whey and whey permeates.

2. Cottage cheese production from ultrafiltered skim milk at 16% solids.

3. Improving the quality of process cheese made from ultrafiltered whole milk cheese base.

4. Role of milk clotting enzymes in cheese curing.


6. Selection and evaluation of proteinase negative cultures in cheese making.

7. Search for sodium-free emulsifiers for process cheese products.

BUDGET FOR 1982-83

<table>
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<tr>
<th>Description</th>
<th>Budget</th>
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<tr>
<td>8 1/2 time stipends @ $4,800</td>
<td>$33,600</td>
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<tr>
<td>Part time labor</td>
<td>4,000</td>
</tr>
<tr>
<td>2 Part time technicians</td>
<td>20,000</td>
</tr>
<tr>
<td>Milk, chemicals and supplies</td>
<td>13,400</td>
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<tr>
<td>Equipment</td>
<td>14,000</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>$85,000</strong></td>
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<tr>
<td>9 Participants</td>
<td>$45,000</td>
</tr>
<tr>
<td>Ag Experiment Station</td>
<td>20,000</td>
</tr>
<tr>
<td>V.P. Research</td>
<td>25,000</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>$85,000</strong></td>
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DERIVATION OF SINGLE CELL PROTEIN AND WHEY DISPOSAL TECHNIQUES UTILIZING RHODOPSEUDOMONAS SPHAEROIDES GROWN ON WHEY AND WHEY PERMEATE

R.S. Reinbold

Photosynthetic bacteria have been employed in Japan as single cell protein sources, organic fertilizers and waste solution purifiers. Kobayashi et. al. determined that the bacterium Rhodopseudomonas capsulata provides 60% protein (d.b.) with an exceptional amino acid ratio. Vitamin, mineral, fat and carbohydrate cellular components are also present in desirable quantities. The organism may also have an hormonal stimulation effect that promotes poultry feeding. When used as organic fertilizer the cellular material affects the growth of Mandarin oranges favorably. Kobayashi also found that waste solution purification expenditures can be reduced greatly when a photosynthetic bacterial pre-treatment is employed.

Research conducted in our laboratory indicates that whey and whey permeate may be utilized as media for the growth of Rhodopseudomonas sphaeroides, a purple non-sulfur photosynthetic bacterium very similar to Rhodopseudomonas capsulata in metabolic capabilities and cellular composition. The goal of our research is to economically derive single cell protein from these organisms while reducing the difficulties and expenditures involved in whey disposal. A proposed research flow chart is attached.

Rhodopseudomonas sphaeroides is capable of growing under diverse modes of metabolism in whey and whey permeate, i.e., photoorganotrophically in the light, aerobically in the dark and microaerophilically in low illumination, but photoorganotrophy is favored. In this type of metabolism, energy is provided by light and an organic substrate serves as both the carbon and electron donor for photosynthesis. Nitrogen-fixation is also favored by photoorganotrophy.
Nitrogen-fixation is desirable since it is a means for the cell to produce its protein requirements without the presence of combined nitrogen sources.

We have found that Rhodopseudomonas sphaeroides grows most rapidly in a 4% solids solution of whey or whey permeate fortified with .1% yeast extract. The yeast extract provides growth factors and nutrients lost during the ultrafiltration process. The 4% solids content is similar to the solids content of liquid whey permeate so one processing step might be eliminated if whey permeate is to be used as a growth medium. The optimum pH for growth is near neutrality, but the bacteria have been observed to grow over a range of 4.0 to 8.5. Over the course of incubation, the pH may only drop one unit. The wide pH range for growth and the small reduction in pH by the organisms may help to remove difficulties in the continuous culture process. The optimum temperature for growth is between 30° and 35° C with very slow or no growth occurring above 40° C. The optimum illumination intensity is 600 footcandles and the atmosphere favoring photoorganotrophy and nitrogen-fixation is a CO₂ - N₂ mixture. A list of Rhodopseudomonas sphaeroides growth characteristics is attached.

At present it is not known what serves as the carbon and electron donor for photoorganotrophy in whey and whey permeate. There is, however, evidence to believe that lactose is not utilized until other forms of substrate have been depleted. A phenol-sulfuric acid qualitative test was used to monitor lactose degradation over the course of incubation. No lactose appeared to be utilized until the turbidity of the culture became great and it appeared that only a small portion of the lactose was ultimately degraded.

On the other hand, in a study of the growth of a mixed culture of
proteinase negative *Streptococcus cremoris* and *Rhodopseudomonas sphaeroides* in synthetic media, the photosynthetic bacteria attained high numbers ($5.6 \times 10^9$ CFU/ml) and all of the lactose was removed. Proteinase negative organisms were chosen for their limited reproductive abilities, but great lactose fermentation capabilities. It is assumed that the lactic organisms converted lactose to lactic acid which was subsequently used as a carbon and electron source by the photosynthetic bacteria. This seems very likely to have occurred since after three days of incubation there was a negligible reduction in pH (6.8 to 6.5). This experiment has not yet been conducted in whey or whey permeate, but is likely that the results will be similar. The implications of success in this type of fermentation are important in that a large population of single cell protein bacteria is attained and lactose is effectively removed.

We have also determined that *Rhodopseudomonas sphaeroides* utilizes lactic acid and fixes nitrogen concomitantly. A synthetic medium without a protein source and lactic acid added as the carbon and electron donor proved to be a satisfactory environment for growth when supplied with a CO$_2$ - N$_2$ atmosphere. The control without N$_2$ gas did not support growth. Growth of the bacterium in whey permeate may substantiate this finding. Whey permeate is very low in combined nitrogen, but relatively rapid growth and large populations have been observed in this medium. Specifically, one colony inoculated into 100 ml of whey permeate supplied with a CO$_2$ - N$_2$ atmosphere produced $2.8 \times 10^9$ CFU/ml within 7 days. Addition of yeast extract may decrease the required incubation time more than three-fold. Under identical experimental conditions, addition of .1% yeast extract can reduce the time necessary to achieve this population size to a total of only 60 hrs.
A study was undertaken to determine whether lactase has any effect on growth rate. No difference was observed, but this is consistent with the findings of other researchers. Glucose can be utilized photoorganotrophically by *Rhodopseudomonas sphaeroides* but growth is normally slow. Growth on galactose has not been cited in the literature.

Cell populations are determined by growth on solid MGR medium. Plates are incubated at 30° C with a CO$_2$ - N$_2$ atmosphere and under an illumination intensity of 600 footcandles. Colonies are countable after 48 hours. The spiral plater yields higher population values than conventional pour or spread plates and has therefore been chosen for this study. A standard cuved depicting growth versus absorbance of liquid cultures has been constructed and is attached.

The research conducted to date is only preliminary, but it shows promise that photosynthetic bacteria may be utilized for single cell protein production and whey disposal.
RESEARCH PROPOSAL

Purified Whey

Single Cell Protein

Cheese ➔ Whey ➔ Ultrafiltration ➔ Whey Permeate

Photosynthetic Bacteria

Retenate

Milk

Sewerable Photosynthetic Bacteria

Continuous Culture

Further Processing?

Purified Whey

Separation Techniques
a. Centrifugation
b. Precipitation
c. Ultrafiltration
d. Separation

Feed

Proximate Analysis

a. Amino acid composition
b. Vitamins, minerals
c. Carbohydrate, fat
d. Crude fiber

Single Cell Protein

Processing
a. Freeze Dried
b. Dried
c. Paste
Desirable aspects of single cell protein production by photosynthetic bacteria

1. Substrate is cheap or cost-free.

2. Waste is efficiently purified reducing capital expenditures.

3. Contamination by other microorganisms is easily controlled.

4. Photosynthetic bacteria provide higher protein contents and better amino acid compositions than most other SCP sources. Also rich in vitamins.

5. Have a more easily digestible cell wall.

6. Palatable to livestock.

7. Are not pathogenic or toxic.

8. Can fix nitrogen gas reducing expensive combined nitrogen requirements.
Composition of general components of photosynthetic bacteria, chlorella, and yeast cells

(g/100g dry weight)

<table>
<thead>
<tr>
<th></th>
<th>Photosynthetic bacteria</th>
<th>Chlorella</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>60.95</td>
<td>55.92</td>
<td>50.5</td>
</tr>
<tr>
<td>Crude fat</td>
<td>9.91</td>
<td>8.07</td>
<td>1.1</td>
</tr>
<tr>
<td>Soluble carbohydrates</td>
<td>20.83</td>
<td>21.04</td>
<td>39.3</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2.92</td>
<td>12.09</td>
<td>2.1</td>
</tr>
<tr>
<td>Ash</td>
<td>5.39</td>
<td>3.28</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Photosynthetic bacteria: *Rhodopseudomonas capsulata*
Chlorella: *Chlorella vulgaris*
Yeast: *Saccharomyces anomalus*
Amino acid composition of the cell hydrolysates of photosynthetic bacteria, chlorella and yeast

(g/100g dry weight)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Photosynthetic* bacteria</th>
<th>Chlorella*</th>
<th>Yeast*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>2.86</td>
<td>2.71</td>
<td>3.76</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.25</td>
<td>1.06</td>
<td>0.90</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.34</td>
<td>3.24</td>
<td>2.50</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4.56</td>
<td>4.74</td>
<td>3.11</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.70</td>
<td>2.28</td>
<td>2.65</td>
</tr>
<tr>
<td>Serine</td>
<td>1.68</td>
<td>2.12</td>
<td>2.75</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5.34</td>
<td>4.62</td>
<td>6.21</td>
</tr>
<tr>
<td>Proline</td>
<td>2.80</td>
<td>2.12</td>
<td>1.77</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.41</td>
<td>2.28</td>
<td>2.18</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.65</td>
<td>2.98</td>
<td>2.86</td>
</tr>
<tr>
<td>Valine</td>
<td>3.51</td>
<td>3.02</td>
<td>3.20</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.58</td>
<td>0.27</td>
<td>0.51</td>
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<tr>
<td>Isoleucine</td>
<td>2.64</td>
<td>2.44</td>
<td>2.63</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.50</td>
<td>4.46</td>
<td>3.54</td>
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<tr>
<td>Tyrosine</td>
<td>1.71</td>
<td>0.96</td>
<td>1.30</td>
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<tr>
<td>Phenylalanine</td>
<td>2.60</td>
<td>2.65</td>
<td>2.20</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.09</td>
<td>0.64</td>
<td>0.66</td>
</tr>
<tr>
<td>NH₃</td>
<td>4.01</td>
<td>2.58</td>
<td>5.30</td>
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</table>

*Photosynthetic bacteria: **Rhodopseudomonas capsulata**

Chlorella: **Chlorella vulgaris**

Yeast: **Saccharomyces anomalus**
Vitamins, pigments, and other elements contents in photosynthetic bacteria cells per 100g dry weight

<table>
<thead>
<tr>
<th>Element</th>
<th>ug/100g</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3,600</td>
<td>N 9.75</td>
</tr>
<tr>
<td>B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>3,000</td>
<td>P 2.49</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2,000</td>
<td>K 0.21</td>
</tr>
<tr>
<td>B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>200–2,000</td>
<td>SiO&lt;sub&gt;2&lt;/sub&gt; 0.82</td>
</tr>
<tr>
<td>C</td>
<td>20,000</td>
<td>Ca 0.87</td>
</tr>
<tr>
<td>D</td>
<td>10,000 I.U.</td>
<td>Na 0.31</td>
</tr>
<tr>
<td>E</td>
<td>31,200</td>
<td>Fe 0.13</td>
</tr>
<tr>
<td>RNA</td>
<td>4.9%</td>
<td>Mg 5.0</td>
</tr>
<tr>
<td>DNA</td>
<td>1.0%</td>
<td>Mn 0.00</td>
</tr>
<tr>
<td>Bacterio-chlorophyll</td>
<td>5.61%</td>
<td>Cu 0.0021</td>
</tr>
<tr>
<td>Carotenoid pigments</td>
<td>4.17%</td>
<td>Zn 0.11</td>
</tr>
</tbody>
</table>
Optimum Temperature - 30-35°C
Atmosphere - CO₂ - N₂ (95% N₂ is optimal)
Illumination - 600 footcandles
pH - 7.0
pH range - 4.0 - 8.5
Medium (non-synthetic) - Whey at 4% solids
Carbon and Electron Donors - Glucose, Fructose, Mannose, Organic acids, Fatty acids, Amino acids etc.

Glutamic Acid 2.00 g
Malic Acid 2.70 g
K₂HPO₄ 10 ml of 80 g/l stock soln.
KH₂PO₄ 0.5 ml of 10% stock soln.
(NH₄)₂HPO₄ 10 ml of 80 g/l stock soln.
MgSO₄ 7H₂O 1 ml of 200 mg/ml stock
MnSO₄ 0.5 ml of 10 mM stock soln.
CaCl₂ 2H₂O 1 ml of 53 mg/ml stock soln.
Nicotinic Acid 1 ml of 2 mg/ml stock soln.
Thiamine 0.5 ml of 2 mg/ml stock soln.
Biotin 0.5 ml of 100 mg/ml stock soln.
Fe citrate 2.5 ml of 9 mM stock soln.
Yeast Extract 1 g
Agar (for solid Medium) 15 g (1.5%)
Adjust pH to 7.2 before autoclaving with NaOH

For Mixed Culture of lactics and photosynthetics, the glutamic acid and malic acid were exchanged with 1.0% Lactose. For N₂ Fixation study glutamic acid, malic acid, (NH₄)₂HPO₄ and yeast extract were omitted, lactic acid at .1% was added and the pH was adjusted to 7.2 before autoclaving.
GROWTH OF *Rhodopseudomonas sphaeroides* IN WHEY PERMEATE

![Graph showing the growth of Rhodopseudomonas sphaeroides in whey permeate. The graph plots the log of CFU/ml versus absorbance at 680 nm. There is a linear relationship between the two variables.](image-url)
References


ELECTION AND PREPARATION OF PAIRED-STRAIN CULTURE STARTERS
FOR CHEESE MAKING
Aly Gamay and Gary Richardson
29 July 1982

Utah State University culture program has successfully been going in cooperating cheese plants for more than two and half years. Cheese quality has been improved and more profit has been realized in those cheese plants.

A workable procedure for the proper selection of bacterial strains has been developed. Such procedure utilizes a computer program and Microtiter techniques which has been developed. If phage appears against one of the strains in the program, a phage-insensitive mutant could be developed and used after examination.

The culture program has recently been modified. Instead of rotating three pairs (composed of five strains), a single pair of lactic strains has been used. Over 1300 vats of Cheddar and Monterey cheese have been made at Cache Valley Dairy Assoc. using this pair exclusively with no loss in vats or low grade cheese due to starter. The isolation of satisfactory strains to assure such acid control without phage problems provides a long awaited breakthrough in lactic culture management. Such programs need further evaluation in USA cheese plants.
The recent discovery of the importance of exclusive use of proteinase negative variants for production of cultured dairy products, suggests additional need for good laboratory methods to isolate and select the best variants available. Furthermore, a satisfactory medium for propagation of these proteinase negative variants is needed.

Quantities of mother cultures used for preparation of bulk stater tanks at cheese plants is another area of investigation. Potential use of freeze dried and frozen concentrated lactic cultures has been examined. It was concluded that mother culture volumes used to inoculate bulk stater tank could greatly be reduced which will minimize the cost of shipping and handling of such cultures. A new system for preparing the mother cultures has been developed and evaluation is underway.
RESISTANCE OF PROTEINASE NEGATIVE STREPTOCOCCUS LACTIS AND S. CREMORIS VARIANTS TO BACTERIOPHAGE AND ANTIBIOTIC ACTIVITY

A.Y. Gamay*, R.S. Hafez, R.J. Brown, C.A. Ernstrom and G.H. Richardson
Utah State University, Logan

Presented at the annual meetings of the ADSA
University Park, PA
29 June 1982 - 2:15 PM

Proteinase positive strains of lactic cultures coagulate milk in 24 hours at 22C, break down casein and are associated with production of bitter flavors in Cheddar cheese. These cultures have been traditionally selected for manufacture of cultured dairy products. Proteinase negative variants grown only to about 20% of the cell numbers of the positive cultures because of the shortage of available nitrogen materials in milk. Their exclusive use has not been advocated because cheese making times are too long. Generally, a freshly isolated single strain culture will have predominantly positive strains but 1 to 2% of the daughter cells become proteinase negative variants. The negative variants build up over a series of transfers until reisolation is required. This occurs when the proteinase positive cells fail to provide sufficient available nitrogenous matter for the increased number of proteinase negative variants.

If a medium, such as those used in pH controlled production of cultures, contains sufficient free amino acid or peptide nutrients then growth of proteinase negative variants is comparable to their positive counterparts. Thus it is possible to uncouple culture growth from acid production; with rapid growth in the bulk culture tank and acid production with limited growth in the cheese vat or final product. If cell multiplication does not occur in the vat then phage cannot replicate, antibiotics are less inhibitory, less casein is solubilized,
the number of organisms present at milling time in Cheddar cheese, for ripening purposes, is constant from vat to vat and there is greater control over rate of acid production. Since growth is not required in the vat, higher cooking temperatures might be useable throughout the cheese making cycle. Growth of proteinase positive cultures is also inhibited when inoculated at high levels. However, they are not inhibited by the lack of available nitrogen matter. Thus there are problems associated with reduced yields and with bitter flavor production when these are used at high levels. We have evaluated advantages associated with exclusive use of proteinase negative cultures and I will report on our work with phage and antibiotics.

Proteinase positive and negative isolates from Streptococcus lactis and Streptococcus cremoris were obtained after propagation on the milk agar and beta-glycerol phosphate medium described by Limsowitzin and Terzaghi. The isolates were selected on the basis of colony size. The proteinase positive cells form significantly larger colonies. Proteinase activity was confirmed by propagation in sterile reconstituted nonfat dry milk. The positive strains coagulated milk in 24 hours while the proteinase negative variants required over 48 hours, some as high as 96 hours. Both cell types remained sensitive to common homologous phage stock solutions. Positive strains were propagated further in sterile nonfat dry milk and the proteinase negative variants were propagated in the same medium fortified with .5% yeast extract.

Sterile nonfat dry milk substrate with .015% brom cresol purple were inoculated with 2% Prt+ or 8% Prt- variants and .25ml quantities dispensed into Microtiter plate wells. These inoculum levels provided comparable acid production rates for the two types of cells. Serial
dilutions of homologous bacteriophage preparations were added to appropriate rows of inoculated milk using manual Microtiter dilutors. The plates were sealed and incubated through a Cheddar cheese temperature cycle. Figure 1 shows the loss of acid production activity associated with phage activity. The Prt- variants retained 97% of the activity of phage-free strains when challenged with $10^5$ plaque forming units per milliliter (pfu/ml). The Prt+ retained only 65% of their phage-free activity with one to ten pfu/ml inoculation. At $10^9$ the Prt+ acid activity was lost but the Prt- cells produced 57% of the uninfected acid production rate. These data represent the means of four lactis and two cremoris strains. The incoming milk supply could be heavily contaminated with phage without affecting acid production. Such high numbers of phage in the milk are highly unlikely. Exclusive use of Prt- variants in Cheddar or cottage cheese manufacture should eliminate inhibition of acid production due to phage activity.

Dr. Shelaih has conducted similar observations in our laboratories on paired versus single strains. Some are plotted in the next two slides (Figure 1A). Activity of paired 73 and 77 proteinase negative strains was taken as 100% at each phage inoculation level since there was no loss of activity in the pair even with one homologous phage inoculated up to $10^9$ pfu/ml. The lower lines represent the activity of Prt+ single strains inoculated with homologous phage virulent against one strain. Note that significantly better activity resulted when the Prt+ strains were paired. The same relationships were evident (Figure 1B) using strains 33 and 8. The different responses of these pairs are indicative of their growth rate and/or rate of phage increase.
A seven-cycle Heap and Lawrence test was conducted on three strains with Prt+ and Prt- isolates. A mixture of composite whey and phage stock solutions was inoculated every day (Figure 2). On the first cycle both types lost activity but the Prt- lost only 16% while the Prt+ lost 31%. On the second cycle the Prt+ lost over 70% activity and testing of these strains was discontinued. However, the Prt- strains increased in activity to over 97% in the fifth cycle. This was probably due to dilution of nonreplicating phage.

Growth and acid production of lactics were also examined in the presence of antibiotics used in the treatment of bovine mastitis. Pencillin, streptomycin or erythromycin were inoculated at from $10^{-9}$ to $10^{-3}$ into milk containing 2% Prt+, 2 or 8% Prt- cells. Changes in pH and generation times were measured after 5 hours at 38°C. Increased antibiotic levels had more adverse effect upon Prt+ than upon Prt- cells as indicated by changes in acid production and generation times (Figure 3). Penicillin affected both variants yet the least effect occurred when Prt- cells were inoculated at 8%. Figure 4 shows that all three antibiotics affected acid production rates with 2%—but not with 8%—inoculation of the cultures.

The inoculum levels of Prt- cells used produced acid faster than desired in some instances. Where pH controlled proteinase negative cultures were propagated in specially fortified whey-based medium a local cheese plant has made tons of normal cheese with an inoculum level only 11% of that of Prt+ strains.

Significant advantages suggest exclusive use of Prt- variants of lactic cultures in the manufacture of fermented dairy products. These include insensitivity to antibiotics and virulent bacteriophage and
their ability to produce acid even when growth is impaired. Current research indicates a potential for the use of such proteinase negative variants in cottage cheese to increase yield from milk solids and in Italian cheese and similar high temperature products higher cooking temperature can be and with Prt- variants, thus shortening cheese making times.
Fig. 1

LOG BACTERIOPHAGE CONCENTRATION (log pfu/ml)

% ACTIVITY

Prt-

Prt+
Figure 1a

Activity (% of control)

Phage Titer (Log pfu/ml)
A cliv. yr.

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Figure 1b
Fig. 2
Figure 3

Figure 4
SUGGESTED RESEARCH ON PROTEINASE NEGATIVE LACTIC CULTURES
Gary H. Richardson
29 July 1982

1. Evaluate the effects of use of proteinase negative (Prt-) variants upon yields of curd in both Cheddar and cottage cheese experiments.

2. Examine evidences of proteolysis in electron micrographs being prepared by Dr. M. Kalab, Dairy Research Institute, Ottawa Canada.

3. Determine the availability of Prt- variants in the high temperature organisms used in the production of Swiss and Italian cheese. Evaluate their presence in strains of Streptococcus ducans.

4. Optimize the whey-based medium for growth of Prt- variants under pH control.

5. Determine the cheesemaking time modifications that are possible through the use of these variants.

6. Evaluate any significant changes in cheese ripening with exclusive use of Prt- variants.

7. Perfect a simple test to evaluate the presence of Prt+ cells in a culture.

8. Determine the advantages of using paired over single variants, if any.

9. Establish a computer controlled bulk culture production system and refine the inoculation requirements for such systems.

10. Confirm the inoculum levels required to provide the desired rates of acid production.
Table I. Effects of temperature on the calculated make time of Cheddar cheese using Prt+ versus Prt- lactic strain UC 171.

<table>
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Table II. Effects of temperature on the calculated make time of cottage cheese using Prt+ versus Prt- lactic strain UC 73.

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ASSOCIATIVE GROWTH OF FOUR PAIRS OF LACTIC STREPTOCOCCI IN 
PH CONTROLLED WHEY-BASED MEDIA

Carol L. Hansen and Gary H. Richardson
Utah State University

Presented at the Annual Meetings of the American Dairy Science Assoc.
University Park, PA
29 June 1982 - 4:45 PM

(Miss Hansen was not able to attend this meeting.)

We have successfully applied the paired strain program used in New 
Zealand to United States cheese plants. Replacement of strains that 
develop bacteriophage problems is simple in a paired strain program. In 
our studies three pairs have been used in rotation for almost two years. Most strains have been replaced with whey derived mutants or phage 
resistant strains as they became available. The acid control and cheese 
quality have been better than when undefined cultures were used. Most New Zealand plants now use only one pair per plant with no rotation.
The Dairy Research Institute has had the fewest complaints ever and 
cheesemakers wonder why it took so long to arrive at the right pair. We 
are evaluating this concept and one trial plant uses only one pair of 
lactic strains for each fill everyday for Monterey and Cheddar cheese. The plant has used this approach for the past 30 days with fewer phage 
problems than when three pairs were rotated.

Additional phage protection and mutual strain stimulation have been 
reasons for retaining pairs in favor of single strain cultures. We 
wanted to evaluate characteristics of pairs and single strains since 
advantages of single strain cultures have been demonstrated in 
Australia.

Several researchers have demonstrated mutual stimulation when 
strains were paired. However, Collins reported strain dominance within
ABSTRACT

Seasonal Variation in the Ability of Milk and Whey to Support Lactic Culture Growth

by

Rick Cameron Norton, Master of Science

Utah State University, 1982

Major Professor: Dr. Gary H. Richardson
Department: Nutrition and Food Sciences

Milk samples from two cheese plants with overlapping milk supplies were collected monthly for one year in an attempt to measure seasonal variation in the ability of milk and whey to support lactic culture growth. Treatments to control and minimize variability of milk or whey were evaluated to optimize stability in starter culture performance.

Raw milk samples were tested for somatic cell counts, activity tests (modified Horrall-Elliker), acid degree values, and total plate counts. Activity (modified Horrall-Elliker) and inhibitory tests were also performed on pasteurized, pasteurized-vacuumized and high heat milk treatments.

Rennet whey (heated and unheated) was collected from raw and pasteurized-vacuumized milk and tested for lactic culture performance by monitoring growth under pH control for 16 h and measuring milliequivalents of neutralizer (NaOH) added.
Lactic culture performance and stability in raw milk was poor in all seasons.

Culture performance in high heat milk was poor, but demonstrated good repeatability.

Pasteurized milk supported good lactic culture performance and stability.

Pasteurized-vacuumized milk provided excellent lactic culture performance and stability throughout the year.

Culture performance during December through March demonstrated the greatest variation. The cultures performed more uniformly during April through August. September was a transition month. Cultures demonstrated uniformity and optimum culture activity during October and November.

Whey substrates without heat sterilization demonstrated similar results to their milk counterparts. Heat treated whey samples showed seasonal variation, but was less than the non-heat treated whey.
two days when one strain produced antibiotics and within two weeks if neither strain produced antibiotics. The advantages for phage protection are best when each strain in a pair is present in approximately equal proportions. If unbalanced, single strains are produced in effect and phage protection is reduced. Collins found 27C to be best for cultivating lactic cultures to assure comparable generation times during mixed growth. Morrison confirmed this for growth of pairs in a pH controlled whey-based medium. Searle found that pairs propagated in pH controlled media performed comparably to those grown in nonfat-dry milk medium (NDM) in terms of stimulation or inhibition of acid production. Dominance was thought to be accelerated in pH controlled medium though he did not measure it. The superior numbers produced in pH controlled conditions is now well established.

Miss Hansen propagated four pairs composed of six strains in both unfortified and stimulant fortified whey substrate. The nutrient-poor whey substrate was to evaluate possible stimulation where proteinase activity was not involved. The strains in the pairs were factory derived mutants of C2 and C7 and strains BA1 and 134. Paired and single strain cultures were propagated in the whey media in test tubes containing 0.015% brom cresol purple indicator. Twenty percent NH4OH was added dropwise upon appropriate color change to maintain the pH around 6.5. Culture numbers were monitored using an aerobic plate count on M17 agar. Individual strain numbers were determined by preparing plates with over 100 distinct colonies per plate. One hundred colonies were individually streaked on M17 agar, twenty colonies per plate using a technique developed by DRI researchers to quantitate strains in multiple blends. A small drop of phage stock solution was centered on
each streak. The phage was homologous for one strain in the pair. Thus it was possible to determine which strain developed the streak and to quantitate both strains. Activity was measured using a Pearce activity test at 37C.

The data were analyzed statistically.

The log of numbers obtained in 12h in the two media are shown in Table 1. The unfortified medium data is tabulated in the top of the table. An F test was calculated testing the difference between the total numbers obtained from one half of the single strains grown separately compared with the totals obtained when paired. The data are means of either three or four trials. Only the first pair demonstrated highly significant increases in numbers in both unfortified and in the fortified medium. None of the acid activity tests demonstrated any significant improvement with any pair in either medium. Note that there were the expected increases in total cell numbers in the fortified over the unfortified whey. Differences in growth or acid production were thus not significantly improved by pairing strains over growing the temperature insensitive strain alone. The temperature sensitive strains produced fewer cells and would require higher inoculum levels to produce comparable acid activity.

Strains C2A, C2B and C7A were "factory derived" variants with phage sensitivities unlike their parent strains. Strains C2A and C2B also had differing temperature sensitivities thus the positioning in the pair where the temperature insensitive strain is listed first.

Each of the pairs was blended in a 50:50 (V:V) ratio and inoculated in stimulant-whey medium at 1% (V:V). This produced an initial ratio which varied as much as 58:42 as shown in Table 2. Note the higher
standard deviation associated with the initial ratio of this pair. This form of blending will not assure equal proportions of cells. It is strain dependent and adequate as in the case of C7A:C2B.

The final ratios were extremely unbalanced where strain 134 was a component. The insensitive strain numbers were 83 and 90% of the total after one 12h incubation period. The advantage of pairing for phage protection would be lost when so unbalanced. The differences in generation times were 26 and 89 min respectively for the unbalanced compared to 10 and 5 respectively for the better balanced pairs. Strain 134 could not compete well with the insensitive strains. Perhaps it needed more nutrient than was provided or was sensitive to some inhibitory material. The extreme standard deviation in generation time when coupled with BAl suggests great variability of the factors involved. Interactions are evident also in that the standard deviations of both BAl and 134 were higher when paired.

Adjustment of the final ratio was attempted by modification of the inoculation volume ratio. Pair C2A and 134 were selected because they had the closer generation times and should have been the easier to adjust. Initial ratios were changed as indicated in the first column of Table 3. The second column data show the difficulty of predicting initial ratios without actual quantitation of the cells. Final ratios were improved as indicated in the third column. However, they did not reach a 50:50 blend even with an initial 5:95 volume combination. The generation times were improved at the 25:75 ratio but showed the greatest differences at the 5:95 ratio. Though fewer in numbers the C2A propagated faster. Though higher in numbers, the 134 propagated more slowly. This is consistent with single strains: When propagated at
very low numbers they divide frequently – at higher numbers they reproduce less often. The degree to which this occurs in a pair must be tempered by the stimulant or inhibitor properties of each strain.

To assure a 50:50 ratio of cells in paired cultures it is obviously better to propagate them separately, quantitate the cell numbers using a rapid spectrophotometric technique and then blend in the proper ratios into the cheese vat. This is impractical and followed in only a very few places. Volume adjustment at inoculation of the bulk culture is the more common practice. This was practical for only two pairs studied. Volume adjustment of one pair was improved but stabilized and did not reach the desired ratio. Perhaps greater inoculum ratio adjustment could have established the desired ratio. However, the techniques required to evaluate such ratios are impractical. A more practical solution is to provide laboratory pre-tested pairs that demonstrate the ability to retain the desired final ratios in fortified medium under pH control. Another is to use one proven, balanced pair as currently being evaluated. Research currently underway suggests that paired strains perform better than single even when phage is not present. Recent visitors from Australia also indicate a return to "factory derived" pairs or triplets instead of single strains.

There is significant evidence now that the fewer the pairs in use, even if only one, the fewer the acid control and phage problems.

We appreciate the financial support of DRINC and members of the USU Dairy Research Advisory Board.
Paired and Single Strain Protease Negative Lactic Streptococci for Cheese Manufacture

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Department of Nutrition and Food Sciences
Utah State University
Logan 84322

ABSTRACT

After propagation in pH-controlled media, pairs of protease negative lactic streptococci produced acid better than the single component strains. Some protease positive pairs remained balanced with 1:1 ratios and others produced 9:1 ratios after one propagation. Acid production by protease negative variants was unaffected by homologous phage (i.e., remained at 100% of uninoculated controls) either when paired or in single strain culture if the total numbers were about four times those of protease positive cells. Single strain cultures of protease positive cells lost significant activity with added bacteriophage. As bacteriophage concentration increased from $10^1$ to $10^9$ plaque forming units per milliliter, the activity dropped from 72 to 1% of the controls. When paired, the rate of acid production improved and was 90 to 62% of protease negative cells as bacteriophage increased from $10^1$ to $10^9$ plaque forming units per milliliter. Antibiotics affected acid production of both cell types, but protease positives were more inhibited. Pairing did not improve performance of either culture for added antibiotics.

INTRODUCTION

Paired strains of lactic cultures are used effectively in the manufacture of Cheddar cheese (7, 14). Most plants in New Zealand and over 40 plants in the United States now use only one pair of lactic streptococci (Robert Lawrence and Reed Ernstrom, personal communication, 1983). Single culture strains have been used in Australia (6). Fewer culture control problems exist when the number of strains is minimal (1, 6, 7, 14, 17). Suggestions that protease negative (Prt-) lactic cultures be used exclusively (13) prompt reconsideration of the need for bacteriophage (phage) control through the use of pairs or culture rotation. This paper compares the performance of paired and single strain cultures when challenged by phage or antibiotics.

MATERIALS AND METHODS

 Cultures

Both Prt+ and Prt- cells were isolated from stock cultures on milk agar medium (13). The Prt+ isolates were propagated in sterile reconstituted nonfat dry milk (RNDM). A 10% solution was prepared, dispensed into 15 X 150 mm test tubes, capped, and sterilized at 121°C for 15 min. The Prt- isolates were propagated in the same medium fortified with 5% yeast extract. All strains were subcultured weekly by inoculating 1% of a freshly coagulated culture into sterile substrate, storing at 5°C, and incubating at 22°C for 15 h prior to use.

Culture Activity Test

Protease negative isolates were propagated singly and in pairs in whey-based medium under pH control (12). The cultures were inoculated at 0.5, 1.5, 2, 5, and 10% into sterile RNDM substrates and incubated in a Pearce activity test (10). Change of pH was determined. Percent inocula required to obtain a change in pH of 1.1 was calculated by the equation $Y = aX^b$ where $Y$ = % inoculum and $X = 1.1$ change in pH. The $a$ and $b$ values were constants for each strain.
Dominance Test

Pairs of Prt+ cultures were propagated together through one cycle in an external pH control system (12). Each strain was inoculated at .5% for 1% total volume, and the substrate was controlled at pH 6 to 6.2 for 12 h at 27°C. The culture was diluted serially to produce slightly over 100 colonies per plate and plated on M17 (16) agar. Twenty colonies were streaked parallel on an M17 plate (5). Phage stock solution, homologous for one of the paired strains, was streaked perpendicularly across the culture streaks. After incubation 48 h at 30°C, the lysed and unaffected streaks were counted, and the percentage of each strain in the pair was calculated.

Phage Filtrates

Homologous phage isolates obtained from cheese plants during evaluation of a defined culture program (14) were prepared in stock solutions containing from $10^8$ to $10^{10}$ pfu/ml by the technique of Heap et al. (5).

Phage Test

Bromcresol purple was added to RNDM at .015% before sterilization and designated BNDM. Each well in one row of a Microtiter plate (Cooke Engineering Inc., Alexandria, VA) plate was filled with .225 ml BNDM previously inoculated either 2% Prt+ or 8% Prt- cultures (13). A .025 ml Microtiter Diluter was filled with phage stock solution and mixed into the first well in a row. The phage stock was diluted decimally to $10^{-9}$ and mixed within the row of inoculated BNDM wells with the diluter. The last well was an uninoculated control. All wells were sealed with cellophane tape, and plates were immersed in a water bath controlled at Cheddar cheese cook temperatures (10). Color changes in the wells were compared after 5 h as indicative of phage or antibiotic inhibition. A 4-mm diameter combination pH electrode (Sensorex, Westminster, CA) was used for pH measurements in the wells. Change of pH of the inoculated substrates was divided by the pH change in the phage-free or antibiotic-free controls and multiplied by 100 to determine percent activity.

Phage Series Test

Three Prt+ and three Prt- variants, isolated from common parent strains, were subjected to homologous phage stock in a seven culture series (4). A .1 ml phage stock and .1 ml homologous phage stock were added to sterile 10 ml RNDM inoculated with .2 ml culture. The inoculated substrates were incubated through a Pearce (10) test, and changes in pH were compared between substrates with and without added phage.

Antibiotic Test

Stock solutions of penicillin G sodium salt, erythromycin, and dihydrostreptomycin (Sigma Chemical Co., St. Louis, MO) of .01 M were prepared in phosphate buffer (2, 9). These solutions were used like the phage solutions for the antibiotic studies.

RESULTS AND DISCUSSION

Culture Activity Test

When Prt- strains were paired prior to propagation in whey-based medium under pH control, acid production activity in RNDM was better in all but one example than when single strains were used (Figure 1). It was not determined whether dominance of the active strain

![Figure 1. Percent (vol/vol) of single and paired protease negative lactic cultures required to produce a 1.1 pH change in reconstituted nonfat dry milk medium in the Pearce activity test.](image)
occurred or if both grew in a balanced condition. Pairs C63/C73 and C63/L33 performed better than either strain alone, suggesting synergism. Conversely, pairs C161/C63 and C320/C73 showed such discrepancy between single component strains that synergism was not suggested. Pair C63/C73 responded in a way that suggested protease complementation. Such prompts additional study.

Dominance Test

A pair of Prt+ strains, C7/C2B, retained 45:48 and 49:51% cell balance after one (50:50) propagation in pH controlled bulk culture. Conversely, pairs of CA2A/134 and CA1/134 produced final ratios of 83:17 and 90:10. When the former was inoculated in 1:20 ratio to reduce dominance the ratio was still 66:34 after one propagation.

Pairs that remain balanced during bulk culture preparation in a cheese factory should be sought. An assay involving a Spiral Plater appears to be a simple and valuable method to identify compatible pairs (15).

Phage Series Test

Mean activities for three single strain Prt+ lactics (UL8, UL21, and UC171) were 69 and 32% of phage-free controls after the first two cycles of the phage series test (4). Means for Prt- variants of the same strains were 84, 86, 86, 92, 98, 97, and 94% through seven cycles. The Prt- cells, in sufficient numbers to produce ample acid, were indifferent to phage and improved in activity as phage was diluted from one cycle to the next.

Phage Test

The lower bars in Figure 2 are means of percent activities of single and paired lactic strains (UL7, UL8, UL21, UL33, UC91, and UC171) when challenged with one homologous phage. The stacked bars represent one standard deviation above the mean. The Prt- cultures experienced no reduction of activity at any homologous phage inoculation although the strains were sensitive to the phage used. Both single and paired strains retained activity indicating no advantage to blending. Cell crowding, resting cells with low growth rates (13), and the need for acid production to meet energy requirements probably accounted for the ability to produce acid and remain indifferent to disturbing phage.

Single strains of Prt+ cells were reduced severely in ability to produce acid as phage concentration was increased (Figure 2). The coefficients of variation were large (16, 34, 48, 43, and 100%), indicating a wide range of effects when one strain was infected. It also suggested differences in infectivity of phage isolates. When pairs of the Prt+ cells were infected with one phage race, activity improved markedly. Coefficients of variation also were reduced significantly (7, 10, 11, 15, and 19%). Improvement of using Prt+ pairs over single strains were highly significant (P=.01).

Paired Prt- strains have no advantage over single strains if cultures can be protected from phage during bulk culture production (11). Both Prt+ and Prt- cells would be affected adversely if phage were present during growth in the bulk culture tank.

Antibiotic Test

Effects of antibiotics upon single and paired strains are summarized in Table 1. Differences among antibiotics were highly significant (2). Both cell types were affected adversely, but
acid production by the Prt\textsuperscript{T} cells was more inhibited. Pairing of either Prt\textsuperscript{T} or Prt\textsuperscript{N} cells produced no significant advantage over single strains.

**CONCLUSIONS**

Pairing of Prt\textsuperscript{N} strains in cheese cultures is of little value unless it can be established that synergism is involved (Figure 1) (3, 8). The use of an active single Prt\textsuperscript{N} variant should be adequate to assure normal acid production in cheese manufacture. The ability to use single Prt\textsuperscript{N} strains (Figure 2) (6) simplifies continuous fermentation as balance problems associated with paired strains (1, 3, 7), multiple strains (8, 17), or build up of Prt\textsuperscript{N} cells in Prt\textsuperscript{T} cultures (13) are eliminated.

Studies are underway to evaluate cheese aging and yield factors associated with Prt\textsuperscript{N} variants.

**ACKNOWLEDGMENTS**

We are grateful to the USU Dairy Research Advisory Board, Dairy Research Inc., Chicago, IL and to AMIDEAST, Washington, DC, for partial funding of this project.

**REFERENCES**


**TABLE 1. Effect of erythromycin (ERY), penicillin (PEN), and streptomycin (STR) on acid production in Microtiter test single and paired Prt\textsuperscript{T} and Prt\textsuperscript{N} cells UC171, UC73, and UL7.**

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\textsuperscript{1}Means of three strains.
MELTABILITY AND RHEOLOGIC PROPERTIES OF A MODEL CHEESE BASE AND PROCESS CHEESE SYSTEM

Paul A. Savello and C. A. Ernstrom

INTRODUCTION

Cheese base prepared from ultrafiltered (UF) whole milk retentate can serve as a substitute for natural cheese in the production of process cheese. The main drawback of cheese base utilization is the lack of meltability of the final product.

UF whole milk retentate and cheese base are complex materials and sufficiently different from natural cheese products. The UF-Cheese Base-Process Cheese project has investigated the melt defect through the use of model cheese base and model process cheese formulations.

MODEL PROCESS CHEESE FORMULATIONS

A model process cheese formulation using 38.5% commercial butter (80.5% fat, 16.5% water, 2.5% NaCl), 24.3% casein (rennet or acid), 2.5% trisodium citrate and 4.5% NaCl-in-moisture was designed. This formulation yielded a process cheese with 52-54% fat-in-dry matter, 21-22% protein and 39-40% water content. Two kg batches were prepared for all tests.

The casein present in cheese base is more similar in structure to acid casein (in which the macropeptide of k-casein fraction is present) than to rennet casein (in which the macropeptide is absent). A model process cheese using acid casein, therefore, more closely resembles the
cheese base-to-process cheese manufacturing scheme.

Whereas the model process cheese using rennet casein exhibited very good melt quality and texture, the acid casein model system had very little or no melt; also, the cheese plug used in the melt test shrunk and had a porous surface. Clearly, the formulation using acid casein as designed would not serve as a proper model in which desired variables (e.g., whey protein addition) could be introduced and subsequent effects measured.

Rennet casein model system

The rennet casein model process cheese system was prepared as follows:

Step 1. Melt butter in cooker at 48-50 °C; add casein, NaCl and emulsifying salt;

Step 2. Heat mix to 65-68 °C;

Step 3. Add sufficient lactic acid in required amount of water to drop the pH to 5.6-5.7;

Step 4. Blend at 65-68 °C for 4 minutes;

Step 5. Cook to 83 °C and hold at this final cook temperature one minute prior to packaging.

Acid casein model system

A technique was designed in which acid casein could be "conditioned" in the cooker so as to produce a model process cheese with sufficient melt quality. This "conditioning" technique is summarized below:

Step 1. Melt butter in cooker at 48-50 °C; add casein, NaCl
and emulsifying salt;

Step 2. Add 80% of required water together with 5 N NaOH solution;

Step 3. Heat mixture to 65-68 C and blend ("condition") at this temperature for 4 minutes;

Step 4. Add sufficient lactic acid in remaining required water to drop the pH to 5.6-5.7;

Step 5. Cook to 83 C and hold at this final cook temperature one minute prior to packaging.

Meltability results

In Figure 1 the values for acid casein represent the volume of 5 N NaOH added to the 2 kg model cheese mix for the "conditioning" step. As the volume of NaOH increased for "conditioning" it was necessary to increase the amount of lactic acid to reduce the pH to the desired range.

Figure 1 indicates that as the level of NaOH "conditioning" of the acid casein increased so did the meltability of the final product. At "conditioning" levels of "55" and higher the meltability of the model cheese system did not increase further. The decrease in meltability from "25" to "35" was repeatedly noticed and is unexplained. The meltability of the rennet casein model system was significantly greater than all acid casein model systems.
The effect of different emulsifying salts on the meltability of acid and rennet casein model process cheese systems was determined. The preparation of the rennet casein model system was the same as outlined previously. The preparation of the acid casein model system was restricted to the "65" procedure. This procedure used 65 ml of 5 N NaOH in the "conditioning" step of the acid casein.

Figure 2 displays the meltability of both model cheese systems using four different emulsifying salts. Disodium phosphate (DSP) effected the largest difference between the rennet and acid casein model systems. The acid casein model system had excellent melt quality and texture while the rennet casein model system did not melt. Tetrasodium pyrophosphate (TSPP) also showed a large difference between acid and rennet casein model systems - the former system melted very well while the latter system had minimal melt. Trisodium citrate (CIT) and sodium aluminum phosphate (SALP) emulsifying salts displayed good meltability in both model systems with the rennet casein model system melting slightly better than the acid casein model system.
EFFECT OF NATIVE WHEY PROTEINS ON MELTABILITY
OF MODEL PROCESS CHEESE SYSTEMS

Freeze-dried whey protein powder was prepared by ultrafiltering and diafiltering a 15% solution of modified whey protein (36% protein). The solution was concentrated and freeze-dried. The protein content (N x 6.38) of the powder was 72.75%.

Freeze-dried whey protein powder was added to the acid and rennet casein model process cheese systems so that levels of 1.5, 3.0 and 4.5% whey protein were present in the final product. A corresponding amount of casein was removed from the basic model system formulation in order to maintain constant total solids. The preparation of the model cheese systems with added whey protein powder differed slightly from the preparation scheme previously outlined. The whey protein powder was added after the blend had been heated to 74 °C and then the mix was heated to cook temperature (83 °C) and held at that temperature for one minute.

Figure 3 indicates that for both model systems the meltability of the cheeses decreased as the whey protein content increased. All acid casein model cheeses had excellent emulsification. The rennet casein model cheeses showed loss of emulsification as the whey protein content increased. The most "oiling-off" occurred in the 3.0 and 4.5% whey protein content cheeses.
Denatured whey protein was prepared by heating a 1% whey protein solution to 85 C for one hour. The solution was ultrafiltered with diafiltered using deionized water. The whey protein solution was concentrated and freeze-dried. The denatured whey protein powder was added to the rennet and acid casein model cheese systems at the same levels and at the same blend temperature as previously described for the addition of native whey protein powder.

Figure 4 indicates that addition of denatured whey protein to the rennet casein model system decreased meltability as the concentration of the whey protein increased. The model cheeses with denatured whey protein melted slightly better than the model cheeses with native whey protein at the 3.0 and 4.5% levels of whey protein.

Figure 5 indicates that addition of denatured whey protein to the acid casein model system decreased meltability as the concentration of the whey protein increased. At each level of whey protein addition there was no significant difference in meltability between cheese samples containing native or denatured whey protein.
Figure 1

MELTABILITY OF RENNET & ACID CASEIN MODEL PROCESS CHEESE

MELT DISTANCE (mm)

RENNET 25 35 45 55 65 75
ACID CASEIN
Figure 2

MELTABILITY OF MODEL PROCESS CHEESE WITH DIFFERENT EMULSIFYING SALTS

ACID CASEIN

RENNET CASEIN
Figure 3

MELTABILITY OF MODEL PROCESS CHEESE WITH WHEY PROTEINS

- Acid Casein
- Rennet Casein

MELT DISTANCE (mm)

WHEY PROTEIN IN PRODUCT (%)
Figure 4

MELTABILITY OF RENNET CASEIN MODEL PROCESS CHEESE
WITH NATIVE & DENATURED WHEY PROTEIN

[Graph showing the meltability of rennet casein model process cheese with native and denatured whey protein. The graph plots MELT DISTANCE (mm) against WHEY PROTEIN IN PRODUCT (%) with two lines representing Native Whey Protein and Denatured Whey Protein.]
Figure 5

MELTABILITY OF ACID CASEIN MODEL PROCESS CHEESE WITH NATIVE & DENATURED WHEY PROTEIN

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Native Whey Protein

Denatured Whey Protein

MELT DISTANCE (mm)

WHEY PROTEIN IN PRODUCT (%)
End Product Pricing of Milk

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The value of milk to a processing plant is determined by the yield and value of products that can be made from it after deducting processing and marketing costs. While this relationship seems obvious it has never been fully implemented by the dairy industry. In some instances, particularly in cheese manufacture, prices paid for milk bear little relationship to the yield of product.

Originally milk was purchased by volume which led to unethical practices such as watering. Then during the early part of this century the butter industry became very important in the utilization of milk fat. Butter produced during the surplus season was stored for distribution throughout the year. The milk was mostly separated on the farm where skim milk was used for feeding livestock and had little commercial value. This created a great need for establishing fat as a basis for setting milk prices.

With the development of the Babcock test in the United States and the Gerber test in Europe, dairy plants were able to rapidly and accurately test producer milk for fat and pay for it accordingly. For many years the dairy industry was strictly on the "fat standard" and little or no economic value was attached to the milk serum or any of its constituents. In some countries milk still is purchased only in terms of pounds of fat delivered.
As margarine made substantial inroads into the table spread market, butter lost its position of strength and was no longer able to completely serve as the "balance wheel" of the dairy industry in stabilizing the value of milk fat. The nutritional and product producing values of the protein and other milk serum constituents were known, but had never been translated to economic values.

With a decreasing influence of milk fat on milk prices several proposals were made to shift a share of the milk value to the serum constituents (2) (4) (6) (12). This required inexpensive rapid and accurate methods of analysis for the constituent or constituents of major concern. In the absence of satisfactory analytical methods, efforts were made to predict solids-not-fat and/or casein from the fat test.

Jacobsen (7) had shown a positive correlation between milk fat and solids-not-fat (SNF) which gave rise to a formula for predicting the SNF content of milk from the fat test (10).

\[
\% \text{ SNF} = 8.27 + 0.4 (\% \text{ Fat} - 3.0)
\]

The Jacobsen relationship between fat and SNF was based on analysis of over 100,000 milk samples, and while satisfactory for averages of large numbers of samples, it never was expected to accurately reflect the fat-SNF relationship in milk from individual cows or even from individual herds. Sommer (10) pointed out that average values for fat-SNF relationships reported by different authors were not in close agreement. Never-the-less some plants wishing to pay directly for fat and SNF have used the Jacobsen formula for predicting SNF.

Fraker and Hardin (6) recognized the inequity of purchasing milk strictly by fat and proposed the establishment of a base price per 100
pounds of milk testing 3.5% fat with a fat differential that was added to or subtracted from the base price for each 0.1% fat above or below 3.5%. Fat differentials were intended to represent the value of 0.1 pound of fat and an accompanying 0.04 pounds of SNF as suggested by Jacobsen (7), or 0.1 pound of fat and an accompanying 0.04 pounds of casein as suggested by Van Slyke and Price (11). Froker and Hardin (6) recognized the weaknesses in the Jacobsen relationship but stated, "Until some practical method is devised for independent measurement of the solids-not-fat or the casein content of milk when making purchases from individual producers, it is believed that the relationships with fat...will need to be used as a basis for payment."

The concept of a base price with a fat differential received wide acceptance in the United States and some European countries. However, as applied by U.S. Federal Milk Marketing orders the fat differentials have come to reflect only the value of the fat and do not generally include values for SNF or casein. This means that the price paid for milk serum is the same regardless of its amount or composition.

The practical effect of this price system has been to encourage the production of low-fat low-solids milk and discriminate against milk with high percentages of fat, solids-not-fat, and protein. This is illustrated in Table 1.

Table 1. Effect of prices paid for milk containing 3.8 lbs. of fat in 3.3% and 3.8% milk priced at $12.50 with a .16 fat differential.

<table>
<thead>
<tr>
<th>Milk (lb)</th>
<th>Fat Test (%)</th>
<th>Fat (lbs)</th>
<th>Milk Price $/100 lb</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3.8</td>
<td>3.8</td>
<td>12.98</td>
</tr>
<tr>
<td>115.15</td>
<td>3.3</td>
<td>3.8</td>
<td>14.03</td>
</tr>
</tbody>
</table>
In this example a producer is $1.05 better off selling 115.15 pounds of milk containing 3.8 lb (3.3%) of fat than 100 pounds containing 3.8 lbs. of fat. It makes no difference to his receipts whether the extra 15.15 pounds is in the form of high solids milk serum or water. In fact, under this pricing system watering can be a very profitable occupation. Even though the 115.15 pounds of 3.3% milk would contain more SNF than the 100 pounds of 3.8% milk, it in no way makes up for the difference in price.

Dairymen have responded to this price system by selecting, breeding, feeding and managing for maximum milk production with little regard for milk composition. It is very revealing to note how few of the top dairy sires in artificial insemination studs have U.S.D.A. ratings with plus PD values for percent fat or percent protein.

This pricing system has served well the needs of the fluid milk industry where the trend has been toward low fat fluid products, and where there has been no economic incentive to increase the solids-not-fat content of milk above the legal minimum. There is evidence that consumers are unwilling to pay for extra SNF or fat in fluid milk.

When used to purchase milk for manufactured products, particularly cheese, the current pricing system has resulted in increasing milk costs per pound of product to the point where some factories have been unable to survive or have had to subsidize their cheese making operations from fluid milk sales.

**COMPONENT PRICING**

Pricing milk by the value of two or more of its individual components has been advocated (2) (3) (12). Milk pricing on the basis
of fat and protein has been most frequently suggested since these two components most closely reflect the economic value of milk (4).

The problem with component pricing has been the difficulty of establishing equitable prices for milk components when the milk is used for different products. For example, the milk protein in nonfat dry milk within the limits of composition of this product, contributes no more to the value of the product that the lactose. Yet in cheese making the protein has great value and most of the lactose has little, and in some cases, a negative value.

Since the output of dairy processing plants is represented by products of various kinds and yields, each of which demands its own price in the market place, it seems that the prices paid for producer milk ought to be based on the value of the products made from it. This would mean that the market place should determine the highest use value for milk of a given composition.

We now have the analytical and computer technology available to carry out this kind of end product pricing.

Our interest in end product pricing was first stimulated by the cheese industry where high milk costs and low yields were creating havoc with a number of factories, especially during summer flush periods. During the summer of 1979, one factory experienced a two week period where they paid more for milk than they received for cheese.

End product pricing is now used by a number of cheese factories, at least one butter-powder plant and more are showing interest each year. Rather than basing milk payments on values of individual components, they are based on the cheese yielding ability of the milk. The basis for this determination is the well known Van Slyke and Price Cheddar cheese yield formula.
\[ Y = \frac{(.93 \ F + C - .1) \ 1.09}{1 - W} \]

where \( Y \) = pounds of cheese per 100 pounds of milk

\( F \) = percent fat in the milk

\( C \) = percent casein in the milk

\( W \) = pounds of moisture per pound of cheese

This formula assumes that 93% of the milk fat is recovered in the cheese and that all of the casein is recovered except .1%. It also assumes that all other milk solids recovered in the cheese plus the salt amount to 9% of the recovered fat and casein.

Barbano and Sherbon (1) and Kosikowski (8) have questioned the accuracy of this formula as it is used in different parts of the country. We have evidence that the form of the equation is quite accurate, and can easily be modified to meet conditions in different plants. Use of this formula in several plants revealed that 90% fat recovery is more common than 93%. When plants have experienced less than 90% fat recovery in cheese, it usually has been traced to cheese making problems.

A direct test for casein that could be run on producer milk is badly needed but is not now available. In the meantime we have suggested using 78% of total milk protein as a casein value. This was the average value for all cows milk reported by Cerbulis and Farrell (5). It also represents the average value for milk from all Holstein cow's. Casein as a percentage of total protein varies from cow to cow but variation in normal mixed herd milk is not large (9).
The working cheese yield formula has been modified to:

\[ Y = \frac{(0.9F + 0.78P - 0.1)}{1 - W} \]

where \( P \) = the percent protein in the milk.

If the fat and protein content of milk are determined, this formula quite accurately estimates the pounds of Cheddar or stirred curd cheese that can be made from 100 pounds of milk. However, if a particular plant finds that its fat or protein recovery is different from that in the formula the constants can be modified to fit the yield. In other words yield prediction can be made to fit what actually happens in any plant.

According to the formula both the fat and casein affect cheese yields independently. However, it is apparent that it takes both fat and casein to make cheese and that there must be a reasonable balance between them in the cheese milk. The standard for Cheddar cheese requires at least 50% fat in the dry matter. This means that the casein/fat ratio in the milk ought to be no higher than about 0.70-0.71. This will give legal cheese with a margin of safety. On the other hand, there is no legal restriction on the maximum amount of fat in the dry matter for Cheddar cheese. Maybe there should be, but there isn't. During the past two decades we have seen the price of cheese increase relative to the price of butter, so much so that it is presently more profitable to sell fat in cheese than as cream for butter making.
EXAMPLE:

If milk tests 3.5% fat and 3.2% protein it should yield

\[ \frac{(0.9 \times 3.5) + (0.78 \times 3.2) - 0.1}{1 - 0.38} \times 1.09 = 9.75 \text{ pounds of Cheddar cheese at 38% moisture} \]

If it tests 3.7% fat and 3.2% protein it should yield

\[ \frac{(0.9 \times 3.7) + (0.78 \times 3.2) - 0.1}{1 - 0.38} \times 1.09 = 10.07 \text{ pounds of Cheddar cheese at 38% moisture} \]

\[ 0.32 \text{ lb. cheese} \times 1.38 = 0.440 \]

\[ 0.2 \text{ lb. fat} @ 1.68 = 0.336 \]

\[ \$0.104 \text{ more profit in selling } 0.2 \text{ lb. of fat in cheese than in butter} \]

These figures suggest that the fat would be worth about 0.52 per pound more in cheese than if sold in cream.

Under present circumstances of prices and cheese standards it is profitable to market as much fat as possible in cheese without reducing the moisture content or quality of the cheese, and without losing too much excess fat in the whey. A casein/fat ratio of 0.64 will give about 55 - 56% fat in the dry matter of cheese and it probably is not advisable to exceed that value, unless you are making low moisture cheese.

The plant must decide the maximum percent fat in the dry matter they are willing to live with. This is then correlated with a corresponding casein/fat ratio in the milk. As long as the casein content of milk is high enough for utilization of all the fat in the milk, the milk value is established strictly from the cheese yield formula.
EXAMPLE:

Yield \times \text{cheese yield value} = \text{price 100 lbs. milk}

9.80 \text{ lbs.} \times 1.28 = 12.54/100 \text{ lbs.}

The cheese yield value represents the amount of money a plant can afford to pay for enough milk to make 1 lb. of cheese. If the fat content of the milk exceeds that dictated by the appropriate casein/fat ratio, the excess fat is subtracted from the total fat in the milk and paid for at a lower rate -- generally a value based on the profitable disposal of whey cream.

EXAMPLE:

The minimum C/F for cheese milk is set at 0.64.

Milk tests 4.4\% fat and 3.4\% protein

\text{Casein} = 0.78 \times 3.4 = 2.65\%

\frac{\text{C/F}}{} = 0.602

\text{Maximum useable fat} = \frac{2.65}{0.64} = 4.14\%

4.40 - 4.14 = 0.26 \text{ lbs. excess fat per 100 lbs. milk}

In this example the cheese yield would be calculated as if the milk contained 4.14\% fat. The excess fat is then valued at its own price and added to the cheese milk price.

EXAMPLE:

\begin{align*}
Y &= \frac{0.9 \times 4.14 + 0.78 \times 3.4 - 1.09}{1 - 0.38} = 11.03/\text{lb. cheese} \\
11.03 \text{ lb.} \times 1.28 \text{ cheese yield value} &= 14.12 \\
0.26 \text{ lbs. excess fat} \times 1.60/\text{lb} &= 0.32 \\
\text{Milk price} &= 14.44
\end{align*}
Determination of the cheese yield value (milk cost per lb. of cheese) must be made by each individual plant. It will depend upon plant efficiency, profitable disposal of by-products such as whey cream and whey as well as the price received for cheese.

If a plant is selling whey cream and dried whey it is useful to calculate the pounds of whey cream fat and pounds of dried whey produced on a PER POUND OF CHEESE basis.

Suppose a plant produces .52 pounds of dried whey and .035 pounds of whey cream fat per pound of cheese. The plant received $.14 per pound for whey, $1.60 per pound of whey fat and $1.38 per pound of cheese.

Receipts per pound of cheese:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Cheese</td>
<td>$1.380</td>
</tr>
<tr>
<td>Whey</td>
<td>.073</td>
</tr>
<tr>
<td>Whey fat</td>
<td>.056</td>
</tr>
</tbody>
</table>

Total receipts per pound of cheese $1.509

Total plant operating costs = $.20 per pound of cheese

You desire $.04 profit per pound of cheese

The cheese yield value would be $1.269.

Cheese yield pricing will pay less for low-fat, low protein milk and more for high fat high protein milk than the current base price-fat differential system.
EXAMPLE:

We may compare the Cheddar cheese yield price with milk priced at $12.50 for 3.5% fat with a .16 fat differential. We will assume the cheese yield value to be $1.28, the extra fat price to be $1.60, the cheese to contain 38% moisture, and the C/F limit to be 0.64.

<table>
<thead>
<tr>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Extra Fat (lb)</th>
<th>Extra Fat Price ($)</th>
<th>Yield (lb)</th>
<th>Cheese Value ($)</th>
<th>Total Milk Value ($)</th>
<th>Old Price ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
<td>8.55</td>
<td>10.94</td>
<td>10.94</td>
<td>11.70</td>
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<tr>
<td>3.2</td>
<td>3.0</td>
<td>0</td>
<td>0</td>
<td>9.00</td>
<td>11.52</td>
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<td>3.4</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td>9.45</td>
<td>12.10</td>
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<td>12.34</td>
</tr>
<tr>
<td>3.5</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td>9.61</td>
<td>12.30</td>
<td>12.30</td>
<td>12.50</td>
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<tr>
<td>3.5</td>
<td>3.2</td>
<td>0</td>
<td>0</td>
<td>9.75</td>
<td>12.48</td>
<td>12.48</td>
<td>12.50</td>
</tr>
<tr>
<td>3.8</td>
<td>3.3</td>
<td>0</td>
<td>0</td>
<td>10.36</td>
<td>13.26</td>
<td>13.26</td>
<td>12.98</td>
</tr>
<tr>
<td>4.0</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>10.95</td>
<td>14.02</td>
<td>14.02</td>
<td>13.30</td>
</tr>
<tr>
<td>4.5</td>
<td>3.3</td>
<td>.480</td>
<td>.77</td>
<td>10.71</td>
<td>13.71</td>
<td>14.48</td>
<td>14.11</td>
</tr>
<tr>
<td>5.0</td>
<td>3.6</td>
<td>.610</td>
<td>.98</td>
<td>11.71</td>
<td>14.98</td>
<td>15.96</td>
<td>14.90</td>
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<tr>
<td>5.0</td>
<td>4.0</td>
<td>.125</td>
<td>.20</td>
<td>13.02</td>
<td>16.67</td>
<td>16.87</td>
<td>14.90</td>
</tr>
</tbody>
</table>

Notice that in this example the cheese yield price and the old price are about the same for milk testing 3.5% fat and 3.2% protein. However, lower solids milk is priced lower than under the old price system and higher solids milk is worth more.

Similar end product pricing has been developed for Swiss cheese and Mozzarella.

For Swiss cheese the yield formula used at one plant is:

$$ Y = \frac{(0.77F + 0.78P - 0.2) \times 1.10}{1 - W} $$

The formula was developed at a single plant from about 80 vat samples collected over one year. Note that the form of the equation is the same as the Van Slyke and Price expression. The constants were developed using a computer and a process called iteration. Several other equation forms were tested by the same process, but none of them improved upon the Van Slyke and Price form.
By the same process a yield formula for part skim Mozzarella and part skim low moisture Mozzarella was developed.

\[
Y = \frac{(.88 F + .78 P - .02) 1.12}{1 - W}
\]

Since Swiss and Mozzarella manufacturing procedures vary considerably from plant to plant these equations should be considered only as guides. Their ability to predict yields in other plants might require modification of the constants.

Both Swiss cheese and Mozzarella are made from milk that is usually standardized by removal of sweet cream. In such cases the milk should be standardized to a constant casein/fat ratio. The Swiss cheese plant under consideration standardizes its cheese milk to an average casein/fat ratio of .86. In this case the computer is programmed to standardize each producer's milk to a \( C/F \) ratio of .86 by removing 40% cream. Yields are then calculated on the standardized milk after removal of the cream. The cheese yield value, which includes the whey fat and whey solids values is then added to the sweet cream value to establish the total milk price.

**EXAMPLE:**

Assume that Swiss cheese contains 39% moisture, the \( C/F \) ratio is .86, the sweet cream price is $1.70 per pound of fat, and the cheese yield value is $1.40.
<table>
<thead>
<tr>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Sweet Cream (lb)</th>
<th>Standardized Milk (lb)</th>
<th>Cheese Yield (lb)</th>
<th>Cheese Value ($)</th>
<th>Sweet Cream Value ($)</th>
<th>Milk Price ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>2.9</td>
<td>.964</td>
<td>99.036</td>
<td>7.33</td>
<td>10.26</td>
<td>.66</td>
<td>10.92</td>
</tr>
<tr>
<td>3.2</td>
<td>3.0</td>
<td>1.250</td>
<td>98.750</td>
<td>7.58</td>
<td>10.61</td>
<td>.85</td>
<td>11.46</td>
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<tr>
<td>3.4</td>
<td>3.1</td>
<td>1.538</td>
<td>98.462</td>
<td>7.82</td>
<td>10.95</td>
<td>1.05</td>
<td>12.00</td>
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<td>3.5</td>
<td>3.1</td>
<td>1.780</td>
<td>98.200</td>
<td>7.83</td>
<td>10.96</td>
<td>1.21</td>
<td>12.17</td>
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<td>3.6</td>
<td>3.2</td>
<td>1.565</td>
<td>98.435</td>
<td>8.09</td>
<td>11.33</td>
<td>1.06</td>
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<tr>
<td>3.8</td>
<td>3.3</td>
<td>2.116</td>
<td>97.884</td>
<td>8.32</td>
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*This is the yield from the standardized milk after removal of cream (it is not the yield per 100 pounds of milk).

In a similar manner, end product pricing can be applied to butter-powder plants. In this case the critical constituents are milk fat and solids-not-fat. The milk may be separated into 40% cream (or any other test) and skim milk. A plant must know the pounds of butter that can be manufactured from each pound of fat purchased (eg. 1.22). It must also determine the percent solids-not-fat in the cream that is recovered in the form of buttermilk, and made available for drying. The computer quickly determines from the fat and SNF in each producers milk its butter yielding capacity, its nonfat dry milk yielding capacity and its dry buttermilk yielding capacity. The plant then sets a butter yield value (the amount the plant can pay producers for enough fat to make 1 lb. of butter), a NDM yield value (the amount a plant can afford to pay producers for enough skim milk to make one pound of nonfat dry milk), and a dry buttermilk value (the amount a plant can afford to pay producers for enough skim milk to make 1 pound of dry buttermilk). If processing costs are known these values may be determined the same way they were determined for cheese. The contribution of each producer's milk to each product is valued and added to arrive at his
milk price. An example of end product pricing for a plant producing butter and nonfat dry milk is illustrated for a situation where butter yield = 1.22 x fat, butter yield value is $1.35, nonfat dry milk yield value is $.78, and dry buttermilk yield value is $.45. Total milk values are compared with traditional pricing at $12.50 with a .16 fat differential.
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End product pricing is an equitable system of establishing the value of producer's milk. It allows prices to be set by the marketplace and encourages the production of milk with a composition best suited to the kinds of products manufactured in a given plant. It is flexible enough to meet the needs of any plant and rewards plant efficiencies in processing and marketing.
REFERENCES


ACCOUNTING IN A CHEESE PLANT USING CHEESE YIELD PRICING

Rodney J. Brown

One of the frustrations of buying cheese milk by the fat differential method is trying to relate the amount of milk purchased to the amount of cheese sold. This is particularly irritating to company accountants. The same amount of money paid for milk never results in the same amount of cheese.

By paying for milk according to its' cheese yielding ability the opposite is true. Knowing beforehand how much of the final sale price is absorbed by the cost of milk allows management to make very accurate financial predictions in spite of variations in milk composition.

The figures shown here demonstrate the economic effects of two different methods of determining purchase price of milk. In the first figure we are looking at the price per hundred weight of milk. The second figure shows the price of enough milk to make a pound of cheese. Most milk falls into the area near where the two lines cross. The horizontal line is cheese yield pricing, with $1.25 representing enough milk to make one pound of cheese.

With such a constant milk cost, accountants can make accurate cost analyses for cheese. The slopes of the increment pricing line makes cost forecasting impossible. Since milk represents by far the largest item in the cost of producing cheese any error in establishing milk cost seriously affects the total cost estimate.
Cheddar Cheese
38% Moisture
3.5% Fat

$12.19 + $.15 fat differential

$1.25 C.Y.V. / $1.40 E.F.V.

Milk Price ($ / 100 lbs)

% Protein
Cheddar Cheese
38% Moisture
3.5% Fat

$12.19 + $.15 fat differential

$1.25 C.Y.V. / $1.40 E.F.V.
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## 38% Moisture Cheddar (40# Blocks)

Using $12.29 \pm .15$

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<td>.004</td>
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<tr>
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<td>- .008</td>
<td>- .008</td>
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| Subtotal | $ .123 | $ .123 | $ .123 | $ .123 | $ .123 | $ .123 | $ .123 |

| Milk | 1.34 | 1.31 | 1.28 | 1.26 | 1.24 | 1.22 | 1.20 |

| TOTAL | $1.463 | $1.433 | $1.403 | $1.383 | $1.363 | $1.343 | $1.323 |
38% Moisture Cheddar (40# Blocks)

Using $1.26 CYV

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Direct Casein Determination of milk using Column Chromatography
R. Carpenter and Dr. R. J. Brown

Several cheese plants presently buy milk according to it's cheese yield. Most of these plants utilize a slight modification of the Van Slyke and Price Formula (figure 1).

Figure 1. Formula to calculate cheese yield

\[
Y = \frac{(.9F + .78P - 0.1)1.09}{(1-W)}
\]

In this formula, casein content of milk is assumed to be 78 percent of total protein. This average value will normally vary from 71 to 87 percent. Casein varies considerably among cows and herds due to such factors as breed, period of gestation, season, disease etc. For example, mastitic milk and colostrum contain a lesser percentage of casein than normal milk. Inserting percent casein in the formula in place of this factor would significantly reduce the variation.

Testing for casein is both difficult and time consuming. First, casein is usually precipitated with acid (pH 4.6, 20 C) as a method of separating it from whey proteins. Then, protein content of the precipitate is measured. Most comonly this is accomplished using Kjeldahl analysis, a test which takes time and uses caustic chemicals.

Other protein tests require the material being analyzed to be dispersed or dissolved in a liquid medium. Therefore, many casein determinations test the protein content of the redissolved casein precipitate. These methods are
difficult to perform accurately and are also slow.

Casein percent can also be obtained by difference. Percent protein of milk minus percent protein of whey gives percent casein by default. Dye binding, fluorometry and formol titration have all been employed for testing protein in milk and whey to obtain percent casein. Nevertheless, this procedure not only doubles the number of protein determinations but also doubles the error term.

All these methods take time. The minimum time for a casein test which employs a precipitation step for separation is about 30 minutes in addition to the protein test. Time is required to titrate milk to pH 4.6, to allow for complete coagulation and then to centrifuge or filter.

Casein can be separated from whey protein without precipitation using size exclusion chromatography. Thus, casein remains in a liquid medium. Milk is passed through a column packed with very small glass beads, each containing numerous uniform pores. Particle size and pore diameter determine the range of separation by size. Large molecules pass through the column around the particles while smaller molecules also pass through the pores, causing them to elute more slowly. Casein micelles are larger than whey proteins so will come out first from a column of this type.

Two peaks were obtained upon elution of milk (figure 2). A Foss Milko Scan has been modified to measure protein in the small quantity of liquid represented in the casein peak. We hope to compare, with high correlation, the protein percent in the pooled sample of this casein peak with percent casein in the milk sample determined using another method.

A long, thin column gave the best separation results. With a flow rate of 0.5 mL per minute through this column, the casein peak came out completely in 5 minutes but the column wasn't ready to accept another sample for a total
Figure 2. Elution peaks of milk

of 10 minutes. This means that one milk sample can be tested every 10 minutes at this flow rate and every 5 minutes at 1 mL per minute. Eventually we will coil the column as in gas chromatography to make it more compact.

We are now studying the effect various solutions have on the casein peak before doing statistical analysis on the method in question. Casein micelles must remain intact during elution so that separation can occur. We will choose the solution that demonstrates the greatest separation at the least cost.
ABSTRACT

Variations in the Coagulation Properties of Milk from Individual Cows.

by

Leslie M. Okigbo, Master of Science
Utah State University.
1982.

Major Professor: Dr. Gary H. Richardson
Department: Nutrition and Food Sciences

Milk samples from fifty individual Holstein cows at the Utah State University Dairy Farm were tested monthly for 10 months for total protein, casein, fat, somatic cells and pH. A new instrument (Foss Formagraph) was used to measure the coagulation properties of the samples. Simulated cheese making procedures were utilized to measure recovery of fat and protein. All the data were statistically analyzed in relation to individual animals, stage of lactation, number of lactation and milk yield.

Significant variations in clotting time and curd firmness were observed in relation to period of lactation, season, individual cow difference, and milk pH. A high, negative correlation (-0.86) was observed between clotting time and curd firmness. The mean clotting time generally increased as lactation progressed and milk yield decreased. This coincided with the change in seasons from Summer, when the cows were generally in mid lactation, to Winter when they
were in late lactation. Curd firmness was generally greater in Summer than in Fall or Winter. Milk samples from 38% of the cows did not coagulate, one month prior to their dry periods. Frequency of the failure of milk to coagulate one month before the dry period was highest in Winter (68.4%) and Fall (31.6%). All samples from cows dried in the Summer coagulated one month before drying. A procedure was developed for measuring the coagulation efficiency of milk from individual cows.

Milk pH was the most significant and independent source of variation affecting clotting time and curd firmness. Stage of lactation correlated significantly and positively with total protein, casein, fat, pH and negatively with milk yield. Total protein, casein, fat, pH and somatic cell counts were highest in Winter, when milk yields were lowest and average days in lactation were longest.

The amounts of casein plus fat that were measured were substituted in the Van Slyke and Price formula to estimate cheese yields during Winter months (January through March). Values ranged from 5.42 to 14.03 lb per 100 lb milk from individual cows with a mean value of 9.18 lb/100 lb milk. Percentages of milk fat plus casein lost in whey ranged from 13.3 to 23.9% with a mean of 17.8% for individual cows.
Changes in Bacterial Population During Ultrafiltration, Diafiltration and Evaporation of Whole Milk Retentate

Charles Gordon Brown

Opportunities for bacterial contamination and growth during the manufacture of ultrafiltered whole milk cheese base are substantial and must be controlled if the cheese base process is to be successful. Experiments were conducted to determine the extent of microbial changes that might be encountered during the process.

A Triclover spiral wound ultrafiltration module containing 4 square meters of filtering surface was used. The feed tank, pump, pipes and UF unit were rinsed four times with deionized water and sanitized with 200 ppm sodium hypochlorite.

Whole milk was pasteurized at 145°F for 30 minutes in a vat pasteurizer. It was then cooled to 122°F prior to ultrafiltration. The pasteurized milk was ultrafiltered until 60% of the milk weight had been removed as permeate. Diafiltration at constant volume was then initiated with deionized water. The water feed tanks and lines had been scrubbed and sanitized with 200 ppm sodium hypochlorite before filling with water. Water drawn from the diafiltration feed tank was plated and showed no visible colony growth after a two day incubation period. An amount of water equal to 38.5% of the original milk weight was used for diafiltration. Diafiltration was followed by ultrafiltration for removal of an additional 20% of the milk weight. A Groen vacuum kettle was cleaned and sanitized with 200 ppm sodium hypochlorite. The interior and exterior of the kettle were rinsed with chlorine as well as the immediate area around the kettle. A chlorine atomizer was used in the sanitizing process. The retenate was divided into two 10 lb batches that were evaporated in the kettle at 38°C and 49°C respectively under
a vacuum of 25 inches. Following ultrafiltration, the first batch was transferred from the UF holding tank to a covered five gallon milk can which had been sanitized with hypochlorite using a spray atomizer. The sample was weighed and immediately placed in the vacuum kettle for evaporation at 38°C. The second sample was placed in a similar, sanitized container and covered until the first sample had evaporated to the desired moisture content. Evaporation in the vacuum kettle required 45 minutes to reach moisture levels of 49.16% and 49.00% at 38°C and 49°C respectively. Using equipment sanitized with 200 ppm hypochlorite samples were drawn from the original pasteurized milk, the retentate prior to diafiltration; the retentate following diafiltration; the retentate following ultrafiltration; and after evaporation.

All samples were diluted with sterile deionized water to a solids concentration of 11.95%; the solids concentration of the original pasteurized milk. This solids concentration was chosen as it seemed to provide adequate fluidity for spiral plating. Samples and dilution water were kept on ice during the entire dilution/plating procedure. Table 1 provides data showing the original sample weight, sample solids content, amount of sterile water added, final sample weight and bacterial counts of the dilute samples. Simple calculations provided the bacterial counts per gram of undiluted sample and are shown in the last column. The bacterial counts were determined using a spiral plater on standard plate count agar. Incubation of plates was for 48 hours at 31.5°C.

Results seem to indicate that evaporation at 38°C provides a favorable temperature for microbial growth while 49°C may be inhibitory. External contamination during the procedure could account for some
of the increase. Shaking of samples during the dilution process could also cause an increase in bacterial counts per plate due to breakage of bacterial chains.

The procedure was repeated four times with fairly consistent results. However, only on the last trial were most of the difficulties worked out. Minimizing microbial growth during the procedure and development of a standard dilution method are goals for future trials.

In these studies, we were mainly concerned with contamination problems. No attempt was made to culture the retentate. Additional studies will examine microbiological changes involving cultured retentate. Survival of desirable organisms as opposed to undesirable organisms will be determined in future research.
TABLE 1

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<th>Sample</th>
<th>Original Sample Wt.</th>
<th>Percent Solids</th>
<th>Grams of Water Added</th>
<th>Weight of Final Sample (11.95% Solids)</th>
<th>Colony Forming Units Per ml. of Diluted Samples</th>
<th>Colony Forming Units Per Gram of Undiluted Sample</th>
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<tr>
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<td>11.95</td>
<td>--</td>
<td>50 g</td>
<td>49</td>
<td>49</td>
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<td>80.27 g</td>
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<tr>
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<td>18.02</td>
<td>68.02 g</td>
<td>70</td>
<td>95</td>
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<td>41.51 g</td>
<td>91</td>
<td>240</td>
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<td>After Evaporation at 38°C</td>
<td>25 g</td>
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<td>81.73</td>
<td>106.73 g</td>
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<td>After Evaporation at 49°C</td>
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<td>82.01</td>
<td>107.01</td>
<td>3,876</td>
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YIELD COMPARISON OF COTTAGE CHEESE FROM CONVENTIONAL AND ULTRAFILTRATION (UF) TECHNIQUES

Ron Raynes

Introduction

Traditionally, cottage cheese has been manufactured in the United States as an acid-coagulated cheese made from skim milk. Lactic starter cultures have been employed in long and short-set methods to produce the needed acid to set the curd. More recently, direct acidification methods have been developed and have met with commercial success in yielding a consistent, high quality product. Since the acceptance of membrane filtration by the dairy industry, several techniques have been proposed for the production of cottage cheese from UF retentates. The main advantage claimed with the UF methods, is an increased yield of 12% above conventional methods. Current research at USU is involved in exploring two procedures of producing cottage cheese from 16% total solids (TS) retentates, and comparing yield and quality with conventionally made cottage cheese. At present, efforts are still concentrating on developing practical make-procedures on a pilot scale to achieve a controlled technique, which must be accomplished prior to examining yield characteristics of the various methods.

Experimental Design

One thousand five hundred pounds of skim milk is vat pasteurized at 145 F for 30 minutes, and divided into three lots of 500 pounds each. The milk is analyzed for TS using a forced-draft oven drying method, and also for protein using the micro-kjeldahl method. One thousand pounds of the skim milk is ultrafiltered to about 18-20% TS using a spirally-wound polysulfone membrane. The retentate and permeate is then analyzed for TS, and the retentate is restandardized to 16% TS. The
standardized retentate is then analyzed for TS and protein. The permeate is retained for further use.

The three lots of skim milk are processed in the following manner: Vat #1 (control) cottage cheese is conventionally made from skim milk with a short-set method, using 2.5% pH-controlled whey based starter. The curd is cut at pH 4.7-4.8 with 1/4" knives, cooked according to the conventional schedule, rinsed with two washes, drained, creamed, and packaged.

Vat #2 cottage cheese is made from 16% TS retentate with a short-set culture method, using 5.0% pH-controlled whey base starter.

Vat #3 cottage cheese is made from 16% TS UF retentate using commercial acidulants, Vitex 750 (liquid phosphoric acid) and Vitex 850 (powdered glucono-delta-lactone (GDL)). The retentate is acidified in the cold state (35 F) with the phosphoric acid to a pH of about 5.5 and then stirred and heated to 90 F, whereupon the necessary amount of GDL is added to reduce the pH to 4.7 and set the curd. In both methods where the cottage cheese is made from retentates, the permeate is heated to 90 F, acidified with phosphoric acid to 4.7, and added back to the vat after the curd is cut. The curds are cooked to a desirable firmness, rinsed, and creamed in the conventional manner. Analyses are made of the curd for TS and protein.

Preliminary Results

Making cottage cheese from retentate involves significant departures from conventional methods of manufacture. For example, coagulated retentate is quite firm and smooth in texture, and requires much less cooking. Conventionally produced cottage cheese is typically cooked to 130-135 F. With retentate curd this final temperature is too
high, and causes shrinking and toughening, resulting in hard rubbery curd. Cooking the curd to a final temperature of 115°F in the last vat processed (cultured method) produced good textured curd.

Levels of rennet can be reduced or eliminated. An optimum level of 0.5 ml/454 kg (full strength cheese rennet) was previously reported by Narasimham for 16% TS retentate. Preliminary results show this may be too high, and a contributing factor to tough curd. The last vat processed used no rennet with good results.

The pH in the vat after cutting can be easily manipulated by adjusting the pH of the permeate prior to adding it to the cut retentate curd. It is well known that the pH in the vat at cutting significantly affects the quality and texture of the curd during the cooking process (D.B. Emmons, Cottage Cheese, p. 40-41).

A procedure for making cultured cottage cheese made from 16% TS retentate has occupied most of our time thus far. Previous research with starter culture activity in retentates has demonstrated a marked inhibition of the acid producing ability of the bacteria below pH 5.0. This inhibitory effect increases as the total solids in the retentate increases, and has been mainly attributed to the accumulation of high levels of acid-solubilized phosphates in the fermenting retentate (R. Narasimhan, Ph.D. Dissertation). Sixteen % TS retentates with normal starter levels were reported to take up to 30 hours to reach a pH of 4.60. Use of concentrated starter cultures overcame the delay in acid production. Therefore, a level of 5% pH-controlled whey base starter was chosen. This required a six hour set, during which the pH was reduced to 4.73 at cutting (See Figure 1). In another vat, 16% TS retentate was allowed to incubate for 21 hours at 85-90°F, reaching a
final pH of 4.61. Coagulation of the cultured retentate was not observed until pH 4.75 was reached.

The pilot production of cottage cheese using direct acidification techniques has not yet been accomplished. Commercial acid levels have been partially determined, and require further research. Sixteen % TS retentate coagulates at higher pH values by direct acidification with phosphoric acid than when cultured at 90 °F (See Table 1). The pH decline of directly acidified 16% TS retentates followed curvilinear relationship with the amount of acid added (See Figure 2). Sixteen % TS retentate can be acidified to pH 5.00 without clotting at 36 °F. However, retentate should not be acidified lower than 5.50, in order to prevent coagulation at 90 °F. This is necessary because the acidified retentate must be stirred and heated to 90 °F, at which point the GDL is dissolved in the retentate. A temperature of 90 °F should be reached for the GDL to hydrolyze and further reduce the pH to 4.75.
Figure 1. pH change in 16% TS retentate during incubation with 5% pH-controlled whey base starter at 90°F.
Figure 2. pH change in 16% TS retentate with addition of phosphoric acid.
Table 1. pH in 16% TS retentate upon addition of various amounts of phosphoric acid

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