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12th Biennial Cheese Industry Conference

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**12th
BIENNIAL
CHEESE
INDUSTRY
CONFERENCE**

August 20-22, 1996

*Department of Nutrition &
Food Sciences*

UtahState
UNIVERSITY

**Western Center
for Dairy Protein
Research and
Technology**

12th Biennial Cheese Industry Conference
August 20-22, 1996
Utah State University

August 20, 1996

7:30 Registration, continental breakfast

8:15 Welcome

THE CHEESE BUSINESS TODAY

Impact of Change on the Cheese Industry

Chair: C. A. Ernstrom, Utah State University

8:30 Bruce Godfrey How the '96 Farm Bill Will Impact the Cheese
Utah State University Industry.

9:15 Richard Merrill Innovation Within the Standard of Identity
Leprino Foods

10:00 Break

10:30 Duane Spomer Codex Alimentarius Cheese Standards
USDA

11:15 Calvin Covington Component Pricing for the Cheese Industry
American Jersey Association

12:00 Lunch

Making a Consistent Quality Product

Chair: Carl Brothersen, Utah State University

1:30 Darold Johnson Importance of Curd and Cheese pH
Cheese Technology Inc.

2:15 Dave McKenna Analyzing Manufacturing Data to Improve Yield
Foss Food Technology Corp.

3:00 Break

3:30 Steve Larsen Using Statistical Processes to Control Product Quality.
Hilmar Cheese Co.

4:15 Mark Johnson Cheddar Cheese as a Specialty Product
University of
Wisconsin-Madison

5:00 Adjourn

5:30 Cheese Smorgasbord Randy Thunell, Waterford Foods
Jeff Miller, Utah State University

August 21, 1996

CHEESE STARTER CULTURES

8:00 Continental breakfast

Strategies for Managing Starter Cultures

Chair: Randall Thunell, Waterford Foods

- | | | |
|-------|--|--|
| 8:30 | Bill Sandine
Oregon State University | Update on New Lactic Cultures. |
| 9:15 | Bruce Geller
Oregon State University | Phage Control in Lactic Acid Bacteria: Summary of Available Techniques |
| 10:00 | Break | |
| 10:30 | Jeff Broadbent
Utah State University | Genetic Modifications of Culture: Where Do We Go from Here |
| 11:15 | Guy Limsowten
Australian Starter Culture
Research Center | Management of Cultures: the Australian Experience |
| 12:00 | Lunch | |

Starter Cultures, Cheese Flavor and Low Fat Cheese

Chair: Craig Oberg, Weber State University

- | | | |
|------|--|--|
| 1:00 | Gerrit Smit
Netherlands Institute
for Dairy Research | Screening for and Control of Debittering Properties of Cheese Cultures |
| 1:30 | Bart Weimer
Utah State University | Culture Metabolism and Flavor During Ripening |
| 2:00 | Jim Harper
Ohio State University | Fatty Acids and an Electronic Nose |
| 2:30 | Jim Steele
University of
Wisconsin-Madison | How to Reduce Bitterness in low-fat Cheese |
| 3:00 | Jeff Broadbent
Utah State University | Off-flavor Production in Cheese |
| 3:30 | Break | |
| 4:00 | Panel Discussion | Cheese flavor: Gerrit Smit, Bart Weimer, Jim Harper, Jim Steele, Jeff Broadbent, Mark Johnson, Don McMahan |
| 5:15 | Adjourn | |
| 6:30 | Steak Fry Banquet Logan Canyon | |

August 22, 1996

CHEESE MANUFACTURING

8:00 Continental breakfast

Novel technologies, cheese making and low fat cheese

Chair: Antonio Torres, Oregon State University

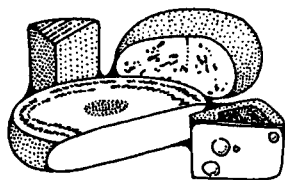
- | | | |
|-------|---|--|
| 8:30 | Antonio Torres
Oregon State University | Application of High Pressure in Cheesemaking |
| 9:15 | Lynn Ogden
Brigham Young University | Use of Homogenization in Cheesemaking |
| 10:00 | Break | |
| 10:30 | Craig Oberg
Weber State University | Manufacture of Fat-free Mozzarella Cheese |
| 11:00 | Bart Weimer
Utah State University | Evaluation/Discussion of Low-fat Cheddar Cheeses |
| 12:00 | Lunch | |

Cheese as a Food Component

Chair: Donald McMahon, Utah State University

- | | | |
|------|--|--|
| 1:30 | Norm Olson
University of
Wisconsin-Madison | Designing Cheeses to Meet Market Needs |
| 2:15 | Charlotte Brennand
Utah State University | Performance of Low-fat Cheeses in Foods |
| 3:00 | Break | |
| 3:30 | Joseph Irudayaraj
Utah State University | Understanding Texture and Moisture in Cheese |
| 4:15 | D. McMahon
Utah State University | Measuring Stretch of Mozzarella Cheese |
| 5:00 | Close | |

12TH
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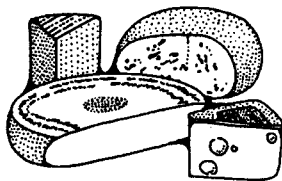
DEPARTMENT OF
NUTRITION AND
FOOD SCIENCES

WESTERN CENTER

Utah State
UNIVERSITY

1. How the '96 farm bill will impact the cheese industry
E. Bruce Godfrey
2. Innovation within the standard of identity
Richard Merrill
3. Codex Alimentarius cheese standards
Duane R. Spomer
4. Component pricing in the cheese industry
Calvin Covington
5. Importance of curd and cheese pH
Darold Johnson
6. Analyzing manufacturing data to improve the yield of a cheese plant
David McKenna
7. Using statistical processes to control product quality
Steve Larsen
8. Cheddar cheese as a specialty cheese?
Mark E. Johnson
9. Further characterization of genetic probe isolated strains of *Lactococcus Cremoris* and their use in cheesemaking
Bill Sandine
10. Phage control in lactic acid bacteria: Summary of available techniques
Bruce Geller
11. Genetic modifications of cultures: Where do we go from here?
Jeff Broadbent
12. Management of cultures: The Australian experience
Gaetan K.Y. Limsowtin

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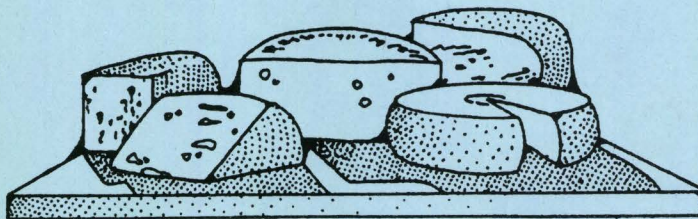


DEPARTMENT OF
NUTRITION AND
FOOD SCIENCES
WESTERN CENTER

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UNIVERSITY

13. Physiological studies leading to the control of debittering properties of lactic acid bacteria in cheese
Gerrit Smit
14. Culture metabolism and cheese flavor
Bart Weimer
15. Swiss cheese flavor - fatty acids and the electronic nose
W. James Harper
16. How to reduce bitterness in low-fat cheese
James L. Steele
17. Off-flavor production in cheese
Jeff Broadbent
18. Application of high pressure in cheesemaking
Antonio Torres
19. Will increased fat surface area help correct quality problems caused by reduction of fat in cheddar cheese
Lynn Ogden
20. Manufacture of fat-free Mozzarella cheese
Craig J. Oberg
21. Designing cheese to meet market demands
Norman F. Olson
22. Performance of low-fat cheese in foods
Charlotte Brennand
23. Effect of aging and biochemical interactions on texture and rheology of low fat cheddar cheese
Joseph Irudayaraj
24. Measuring stretch of Mozzarella cheese
Donald J. McMahon

**HOW THE '96 FARM BILL
WILL IMPACT THE
CHEESE INDUSTRY**



**E. BRUCE GODFREY
ECONOMICS DEPARTMENT
UTAH STATE UNIVERSITY**

HOW THE '96 FARM BILL WILL IMPACT THE CHEESE INDUSTRY

E. Bruce Godfrey
Economics Department
Utah State University

The U.S. federal government has had a major influence on agricultural production and prices, including milk, for over 50 years, but passage of the *1996 Farm Bill* will alter this influence in the future. The title of the bill is indicative of the changes that are likely to occur—*Federal Agricultural Improvement and Reform Act of 1996 (FAIR)*. Since the original farm legislation was drafted more than a half century ago, there has been no piece of legislation that was designed to *reform* and change the orientation of agricultural policy more than this bill. Most farm bills are designed to either reduce production (e.g., acreage reduction programs) or provide subsidies in the form of government purchases of commodities. The passage of *FAIR* represents a major departure from this general policy. The provisions of *FAIR* clearly place more emphasis on market forces. This will have profound impacts on dairy producers and processors.

Several provisions of *FAIR* will have an influence on the dairy industry. The provisions specifically designed to directly address the dairy industry include:

1. the elimination of dairy price supports,
2. provision of recourse loan,
3. consolidation and reform of the milk marketing orders,
4. provision for a northeast interstate dairy compact,
5. provision for a dairy export incentive program (DEIP), and
6. provision for export trading companies.

However, these provisions are not the only things that will affect the dairy industry in the future. There are other provisions in the bill that will indirectly affect dairy industry.

Any discussion of *FAIR* and its influence on the cheese industry must be centered on three general areas. First, the cheese industry is only one segment of the dairy industry. As a result, anything that affects any part of the industry (dairy producers, milk processors, etc.) will also affect the cheese industry. Second, *FAIR* contains provisions that will affect other segments of agriculture in America. Many of these impacts will indirectly affect dairy producers and

processors. Third, as indicated above, *FAIR* represents a major change in farm policy from that which existed in the past.

Major Provisions of FAIR Affecting Dairy

A review and analysis of all of the provisions in *FAIR* are beyond the scope of this paper. The major provisions of *FAIR* that will primarily affect the dairy industry give some indication of the changes that are likely to occur.

Price Supports

The data shown in Figure 1 show the average price of milk received by dairy farmers in the United States from 1970 through 1994. Two things are clear from these data. First, milk prices rose steadily until 1981. After that time, price increases have been relatively constant. Secondly, prices have become less stable and predictable since 1988. One of the major reasons for this change is shown in Figure 2. These data indicate that since 1987 the farm price has been above the government support price. As a result, market, and not government, support prices have been the primary force that has dictated what price dairy producers receive for milk. This same general pattern is also true for cheese (Figure 3). For example, the Commodity Credit Corporation (CCC) price was commonly above or very near the price for cheddar cheese on the National Cheese Exchange (NCE) until 1989. Since that time, the support price has generally been lower. The same cannot be said for butter (Figure 4)—support prices have commonly been higher than spot cash prices reported by the Chicago Merchandise Exchange (CME). As a result, the government has remained a major buyer of butter until the last couple of years when butter prices have generally been higher than the CCC support price. For example, USDA data (*Livestock, Dairy, and Poultry Situation and Outlook*, 23 July 1996) indicated that the wholesale price of Grade A butter was nearly \$0.70 a pound in June 1995, while the support price was \$0.65. The difference is even

greater at the present time—the price of butter in June 1996 was \$1.2925 a pound, which is almost double the support price of \$0.65 a pound.

One of the major provisions of *FAIR* is the elimination of price supports for essentially all commodities. For example, milk price supports are to be reduced according to the following schedule:

<u>Year</u>	<u>Support Price (\$/cwt.)</u>
1996	\$10.35
1997	10.20
1998	10.05
1999	9.90
2000 and after	None

It should be noted that milk prices are currently above the support price. The elimination of price supports represents a change in policy—there will be no minimum price for milk that is supported by government. As a result, the potential for downside price risk for milk producers is enhanced under *FAIR*. This may be viewed favorably by milk processors, but it must be remembered that price supports will also be eliminated for other dairy products (cheese, butter, and dry milk).

Loans

The elimination of price supports for milk in the year 2000 also coincides with the implementation of a loan program. The CCC has historically purchased butter, cheese, and dry milk whenever the market price of these commodities have dropped below the support price. When market prices have been higher than support prices, government stocks have been sold. The results of this action are shown in Figures 5 and 6. These data show that starting in 1988, the amount of CCC cheese in storage was reduced to nearly zero and has remained at low levels since that time. Butter purchases and the amount in storage increased during the early 1980s but has subsequently dropped. As a result, the amount of butter in government storage is low at the present time.

The historic purchase program for butter, cheese, and dry milk is to be eliminated. This program is to be replaced by a loan program for processors that have cheese, nonfat dry milk (NDM), or butter in storage. This loan rate is set at \$9.90 per cwt. This represents a loan by the government for inventory in storage and may be used by processors who are not able to obtain

credit from other sources that is less costly. But sales to the government will no longer occur by the year 2000 unless the provision of *FAIR* are changed. As a result, the federal government will no longer be a major player in determining the minimum price of cheese. The effect of this change, like the elimination of the support price for milk, is to remove the floor on the price of manufactured products. It also means that the storage of stocks of butter and cheese will be controlled by private firms instead of by the government.

Marketing and Pricing

The one area where there is more uncertainty than any other concerns the consolidation of the milk marketing orders. At the present time, there are 33 milk marketing orders (Figure 7). *FAIR* requires the Agricultural Marketing Service (AMS of USDA) to reduce this to no less than 10 and no more than 14 orders by 1999. This will cause a change in the structure of milk prices in most areas and regions. For example, price differences in February 1996 in the various orders varied from a high of \$17.09 per cwt for class I milk in southeastern Florida to \$13.06 in the upper mid-West. One of the major reasons why these differences exist stems from the wide variation of milk production in an area that is utilized for class I production. For example, the percentages varied from a low of 8.3% in the southwestern Idaho/eastern Oregon order in 1995 to 92.4% in southeastern Florida. While differences in class I utilization and prices between orders will probably continue to exist, the smaller number of orders will cause milk prices to be essentially uniform over a larger area. This will, in turn, affect the locations where growth and declines in milk production will occur. It is not known at this time what orders will exist after the number of milk marketing orders is reduced, but, it is clear that changes will occur that will affect the supply of milk in a region or order and the price processors pay for milk.

FAIR authorized the continued existence of the California order—the only state-administered milk marketing order. This maintains the higher California standards for nonfat solids, but it is likely that these provisions will be subject to legal challenge. This may cause

California producers to consider joining the federal system. If California chooses to enter the federal milk marketing order system, a separate order for California is authorized by *FAIR*.

New England dairy industry leaders also sought and obtained authorization to establish a compact or agreement that would be designed to increase New England region producer returns. It is not clear that this authorization will be used to form a compact. As a result, this provision may be a moot issue. Furthermore, it is unlikely that this will have much of an impact nationally even if a compact is formed.

If the change in the milk marketing orders was not enough, Secretary Glickman also charged AMS to develop a new basic formula price to replace the M-W price series that has existed for several years. A new basic formula price (BFP) was developed, but a recent AMS study indicates that the new BFP was pricing class III milk at "30 cents per hundredweight below what the old M-W price would have done" (*Dairy Herd Management*, July 1996, page 36). As a result, it is likely that further refinements will occur. All of the above suggests that price formulas used to set the price paid for milk will become more uncertain in the near future.

Exports

The passage of GATT and NAFTA marked a new era in trade between countries. The basic thrust of these acts was to remove trade barriers and encourage imports and exports between countries. These will tend to make world rather than domestic prices the standard. The passage of *FAIR* is one other piece of legislation that is designed to encourage world trade.

One of the primary thrusts of *FAIR* is the desire to encourage exports. Support prices have generally resulted in prices for milk products that have commonly been higher than the world market prices. When support prices are removed in the year 2000, this barrier to world trade will be removed, but this will not affect all dairy products equally. For example, the domestic prices of butter and dry milk, until very recently, have been below the international price while cheese prices have been higher. As a result, the United States has been a net exporter of butter and dry milk and a net importer of cheese (Figures 8-10). The elimination of price supports will result in prices that

are dictated by world rather than domestic markets with an expected decrease in domestic cheese prices. This will reduce imports and make U.S. manufactured cheese more competitive in the world market. Cheese production and marketing will therefore become increasingly international.

This international orientation is to be encouraged by three provisions in *FAIR*. First, a National Dairy Promotion and Research Board is authorized. This board is authorized to spend check-off monies to develop international markets. Second, the Dairy Export Incentive Program (DEIP) is authorized to be fully funded at the GATT maximum levels. Third, the Secretary of Agriculture is mandated to establish a dairy export trading company. It is not clear as to how these provisions will be implemented, but it is obvious that the dairy industry is being asked to be a major player in the world market, especially in the year 2000 and beyond when support prices are removed. Some of the predicted impact on prices are shown in Table 1.¹

Crop Policy

The preceding provisions of *FAIR* are the ones most commonly viewed by the dairy industry. The provisions concerning crop production could potentially have just as large of an impact as some of these provisions, especially on dairy producers. One of the major parts of *FAIR* was the separation of payments from crop production decisions. Transition payments authorized under *FAIR* provide income support for farmers that is not tied to production decisions. Farmers now have almost total freedom to plant anything that is desired. As a result, lands that have been

¹Anyone who is familiar with the prices that exist today should recognize that the prices predicted for 1996 are significantly different than what exists today. For example, the price of corn is generally \$2.00 higher today than predicted but this price will likely be in the \$3.50 range by fall 1996. In addition, milk prices are higher than those predicted, and butter prices are about double those that were predicted.

Table 1. Predicted Prices for Selected Crops and Dairy Products, 1994-2002

Year	Corn (\$/bu.)	Hay (\$/ton)	Milk (\$/cwt.)	Butter (cents/lb.)	Cheese (cents/lb.)
1994	2.26	84.00	12.97	67.37	131.45
1995	3.05	78.85	12.78	75.55	132.78
1996	2.75	76.72	12.89	75.20	133.56
1997	2.46	76.69	12.87	72.28	133.69
1998	2.31	73.77	12.78	70.07	133.06
1999	2.23	65.39	12.73	69.70	132.79
2000	2.29	64.72	12.31	69.10	129.51
2001	2.33	63.87	12.41	67.95	130.33
2002	2.43	63.70	12.55	68.05	131.75

Source: AFPC Working Paper 96-4, Texas A&M University.

set aside as part of the various acreage reduction programs will come into production. This resultant increase in production will lead to lower prices, enhanced exports of crops, and reduced production costs for dairy producers.

Anticipated Adjustments

Reliance on market forces will also affect the structure of the industry. For example, historic trends (see Figures 11-14) indicate that milk production and production per cow in the U.S. have increased over time while the number of dairy cows and dairy farmers has declined. These trends will probably continue. This same general pattern¹ of fewer but larger milk processing plants will probably also occur.

Perhaps one of the most difficult problems associated with marketing in the dairy industry will occur as a result of eliminating support prices. Dairy producers and milk processors will now be faced with risks associated with decreasing prices that have not been experienced in the past.

¹Data on plant numbers are not readily available.

This will force most dairy producers and handlers to carefully consider practices and alternatives that can transfer some of this risk to others. For example, the use of futures markets will probably become more common.¹ Considerable promise exists for cheese producers to not only use these markets to manage price risk, but it also provides an opportunity for processors to forward contract with producers for milk and use the futures market to offset these risks. This will be especially true in areas where the number of producers declines as a result of consolidation of operations.

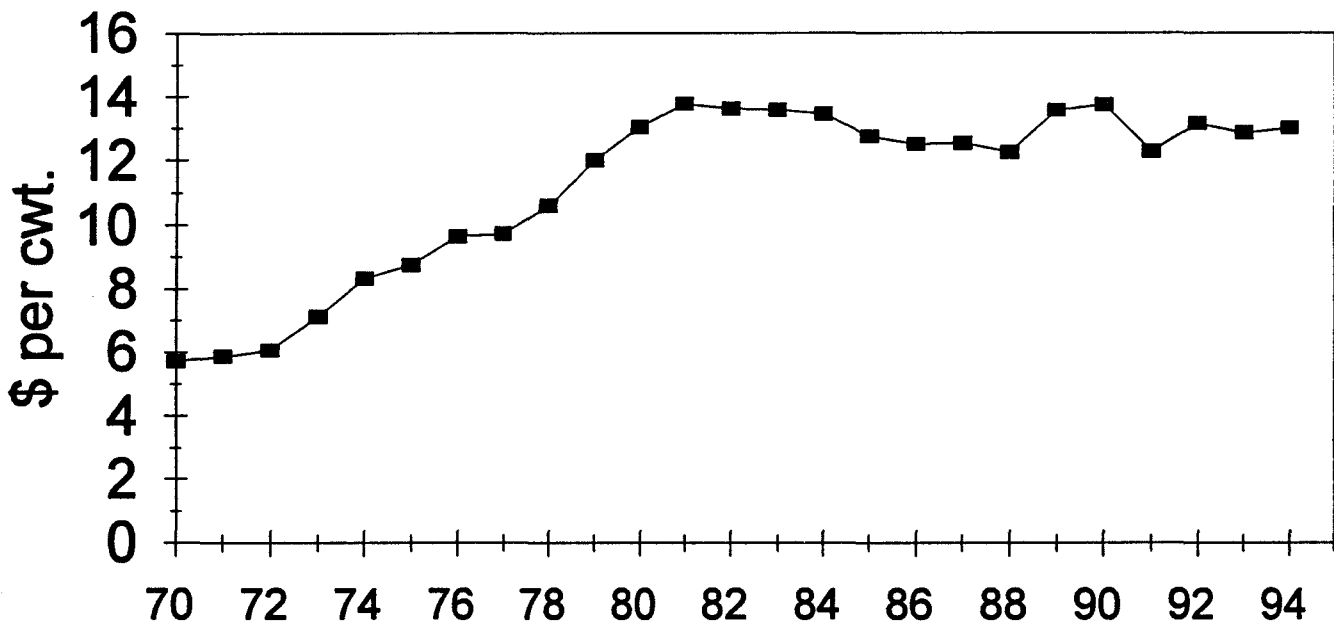
When the federal government no longer purchases butter, cheese, or dry milk, the private sector will carry all stocks. The data in Figures 15 and 16 indicate that butter stocks were probably at an all-time low on 31 December 1994, while cheese stocks have been fairly stable since 1990.² Opportunities will probably exist for profits from storage. This may be especially true if futures markets are used to reduce the risk of changes in price and storage costs between months (arbitrage).

As can be seen from above, passage of *FAIR* will affect the dairy and cheese industries. Most of the market forces have been in operation for at least five years. As a result, the changes will not be dramatic in the short run. The turn of the century (year 2000) will be the year the safety net will be removed for farmers and the dairy industry. As a result, market (price) risk will increase. Some firms will not be able to manage these increased risks and will leave the industry, but it will also provide rewards for those who can manage production and marketing decisions in an increasingly risky world market.

¹Futures markets for milk, cheese, and butter probably would not have been introduced if support prices had been closer to market prices.

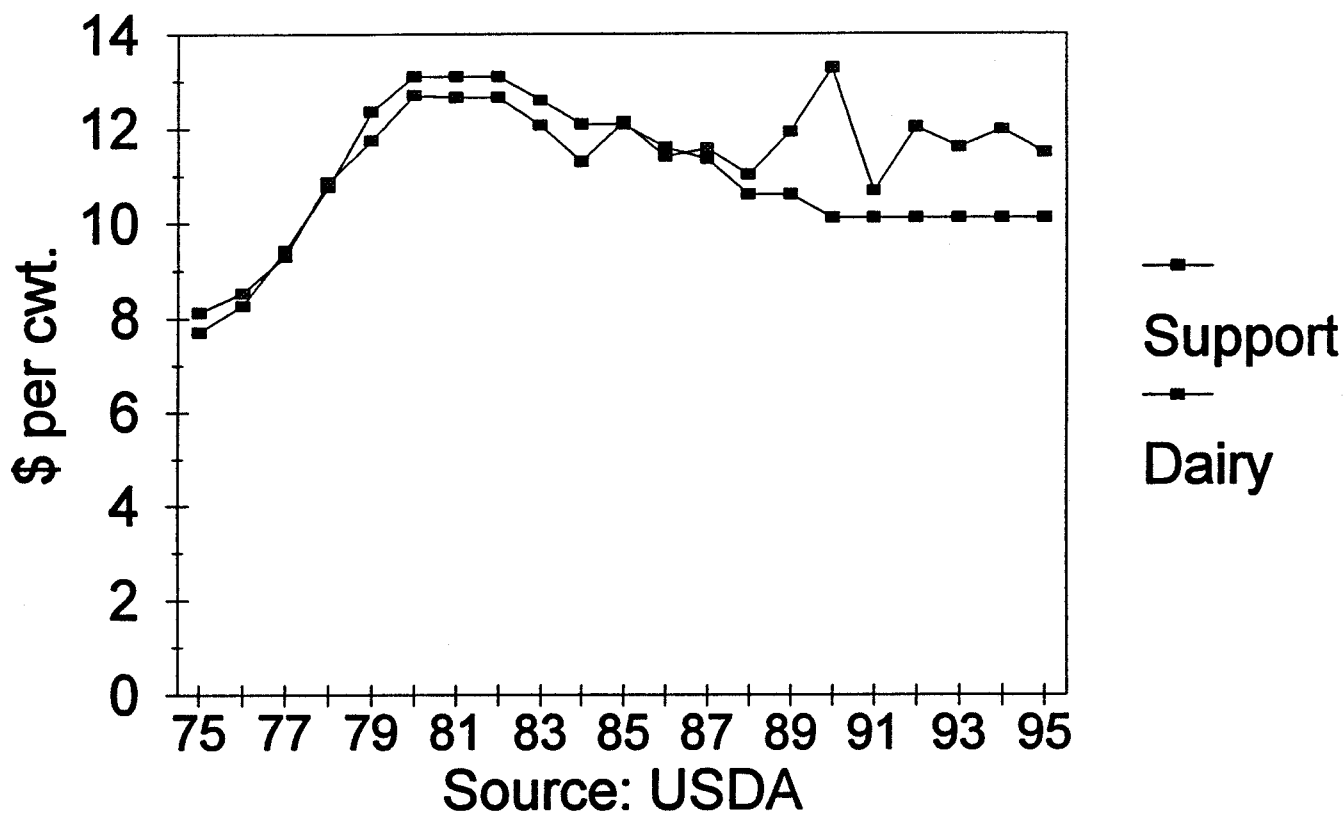
²I have not been able to determine the amount of butter, cheese, or dry milk held by the federal government from the data available to me. All the data I have seen, however, suggest that almost all stocks are being held privately at the present time.

Milk price received by U.S. farmers 1970-1994

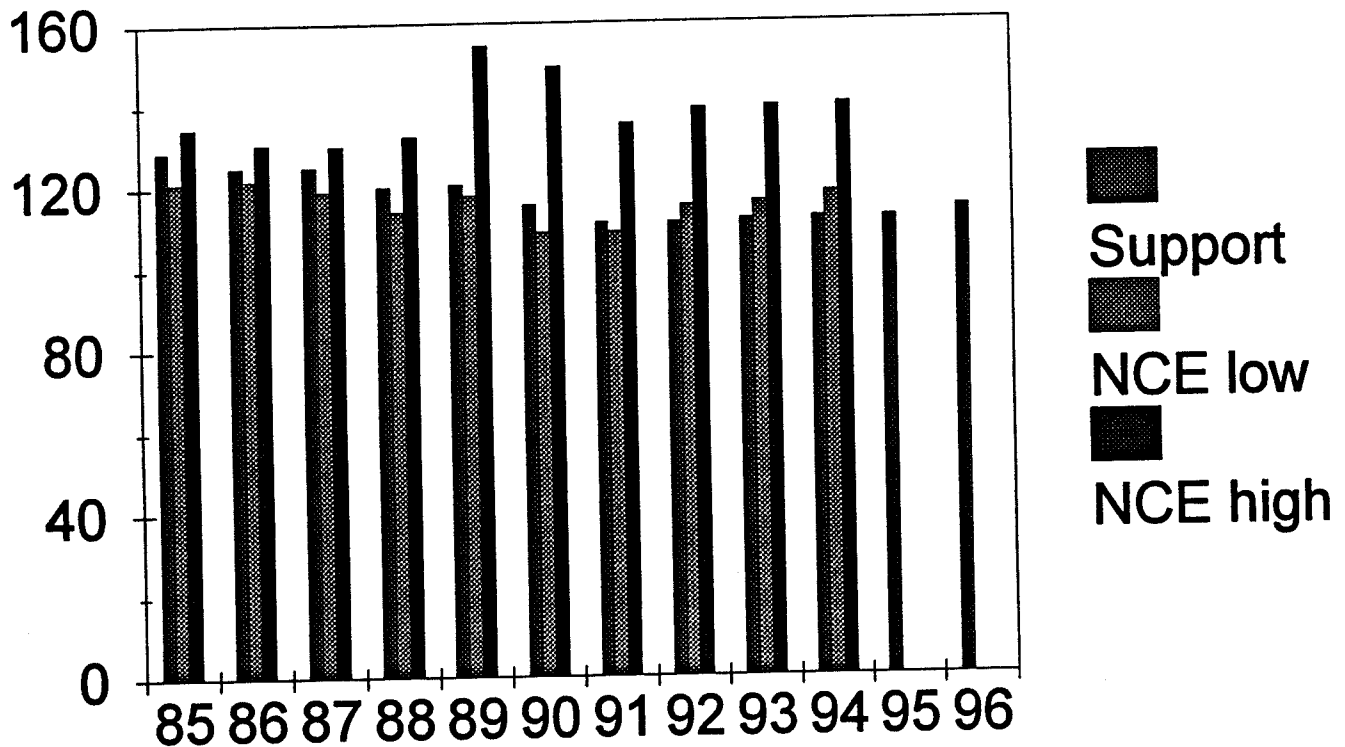


Source: USDA

Support and average milk price received by dairy farmers, 1975-95

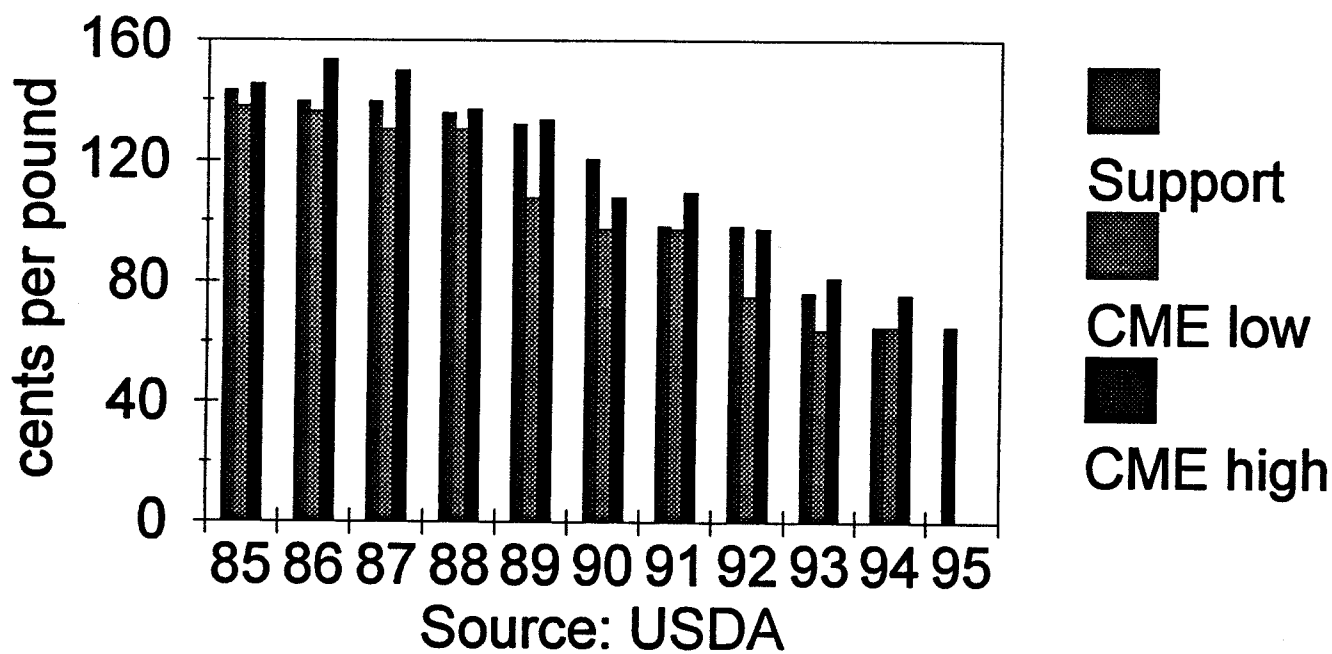


CCC support price and Cheddar cheese prices (NCE 40 lb blocks), 1985-96

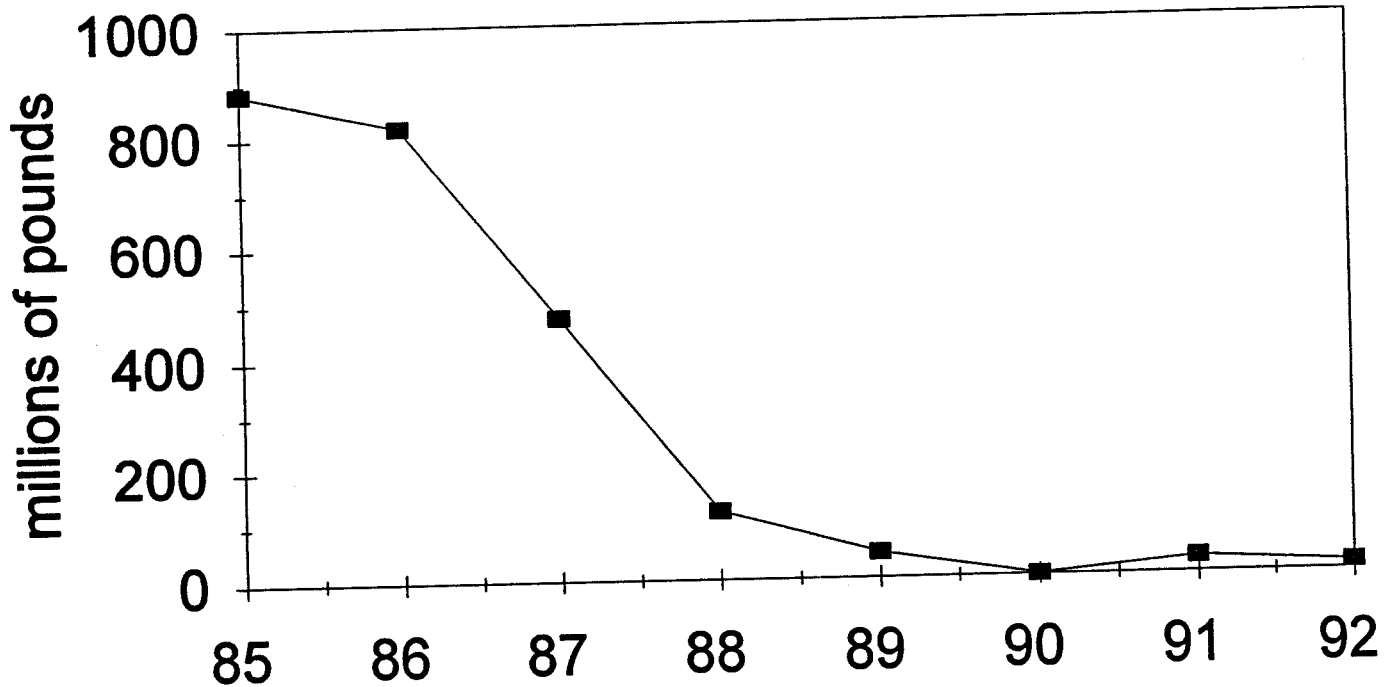


Source: USDA

CME high & low prices and support price for butter, 1985-1995

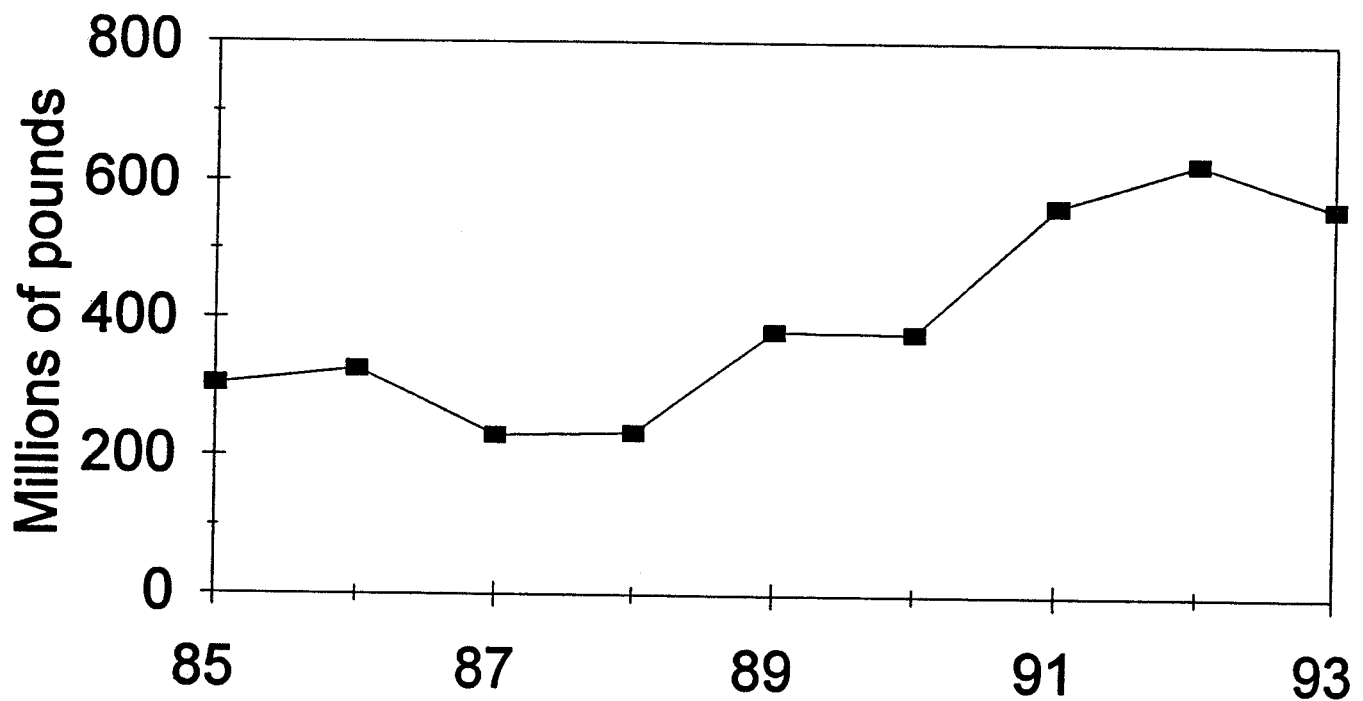


CCC cheese owned on 31 December 1985-1992

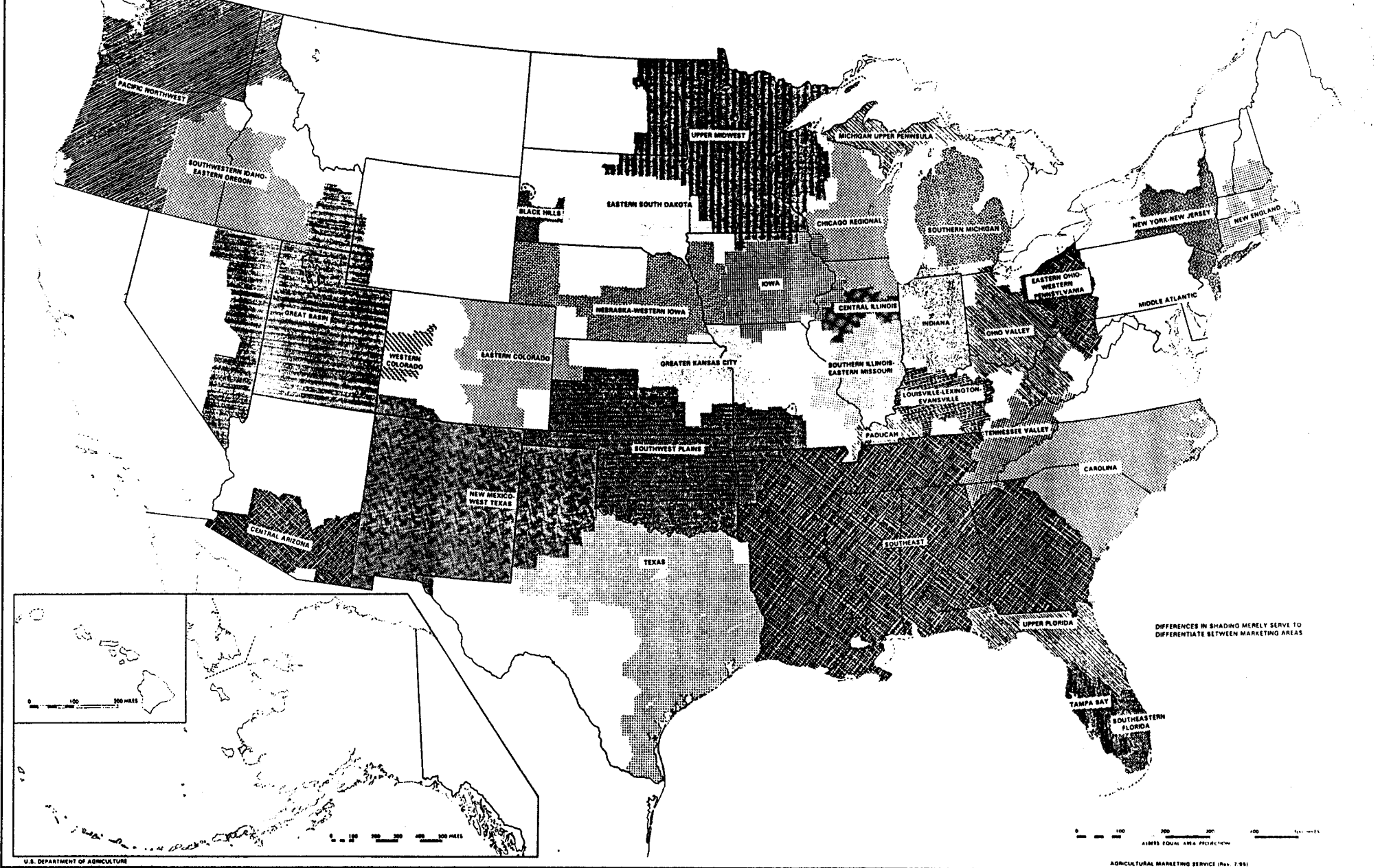


Source: USDA

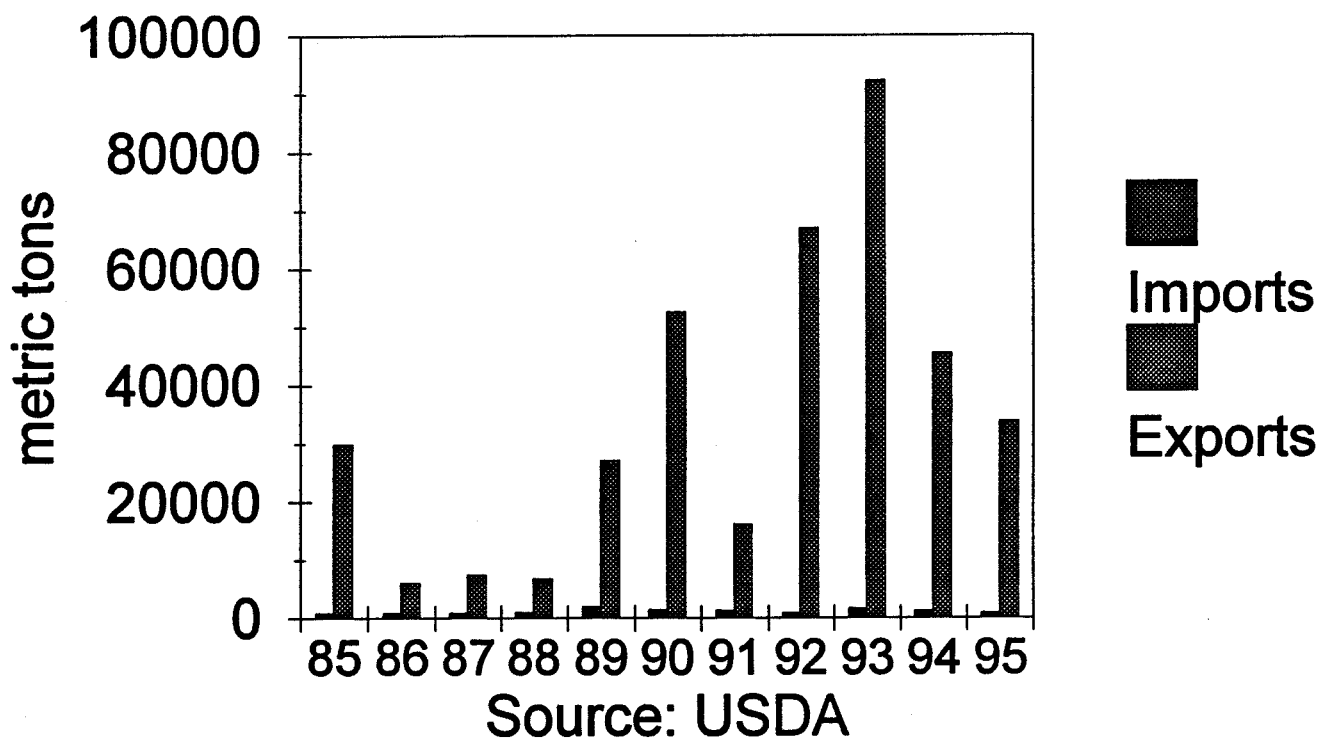
CCC storage of butter & butter oil on 31 December 1985-1993



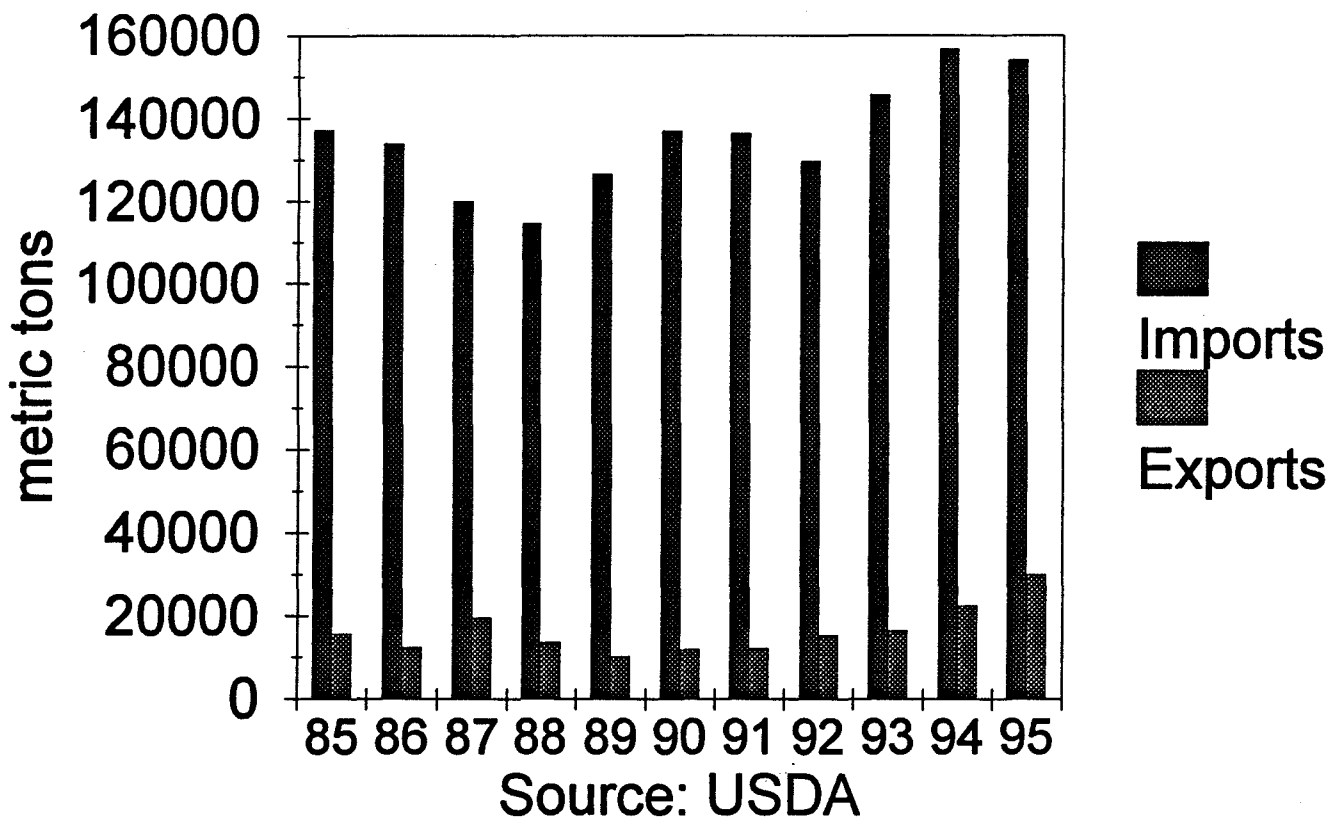
MARKETING AREAS UNDER FEDERAL MILK ORDERS AS OF JULY 1, 1995



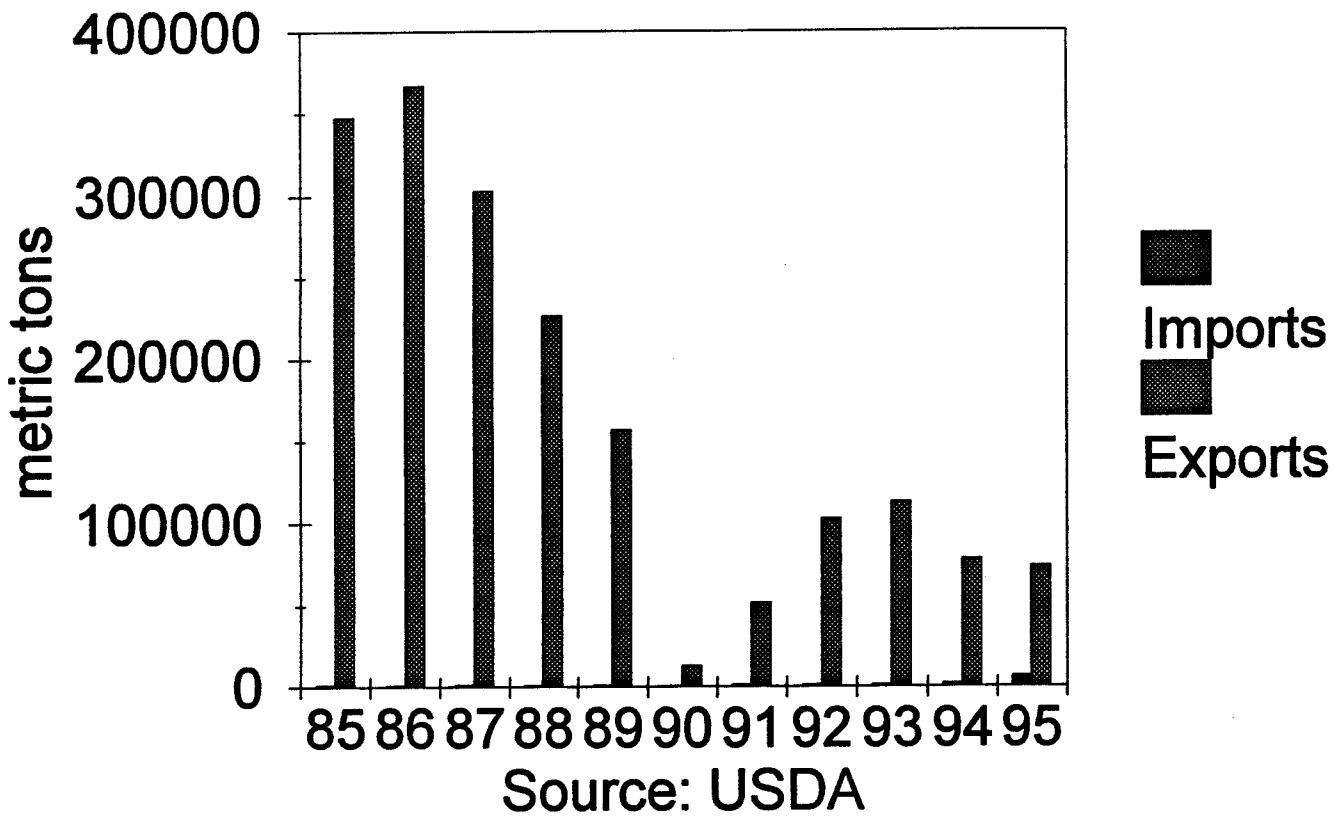
U.S. butter imports and exports 1984-1995



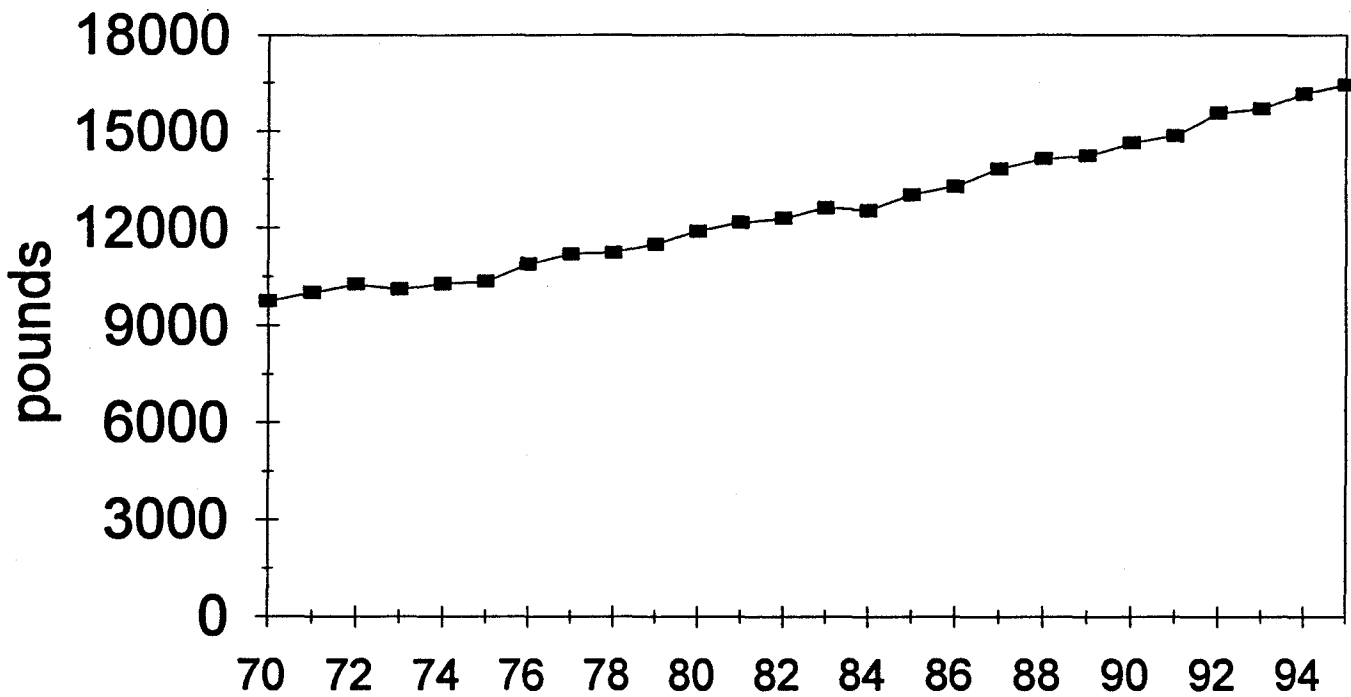
U.S. cheese imports and exports 1985-95



U.S. dry milk imports and exports 1985-95

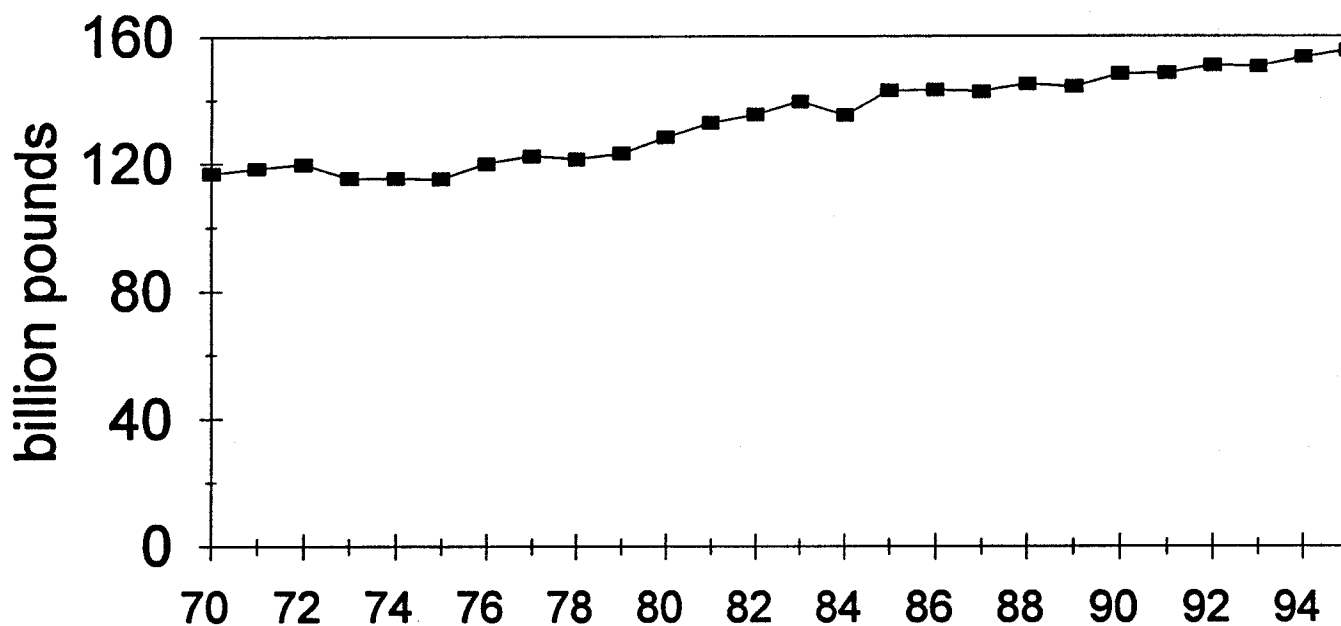


Milk production per cow in U.S. 1970-1995



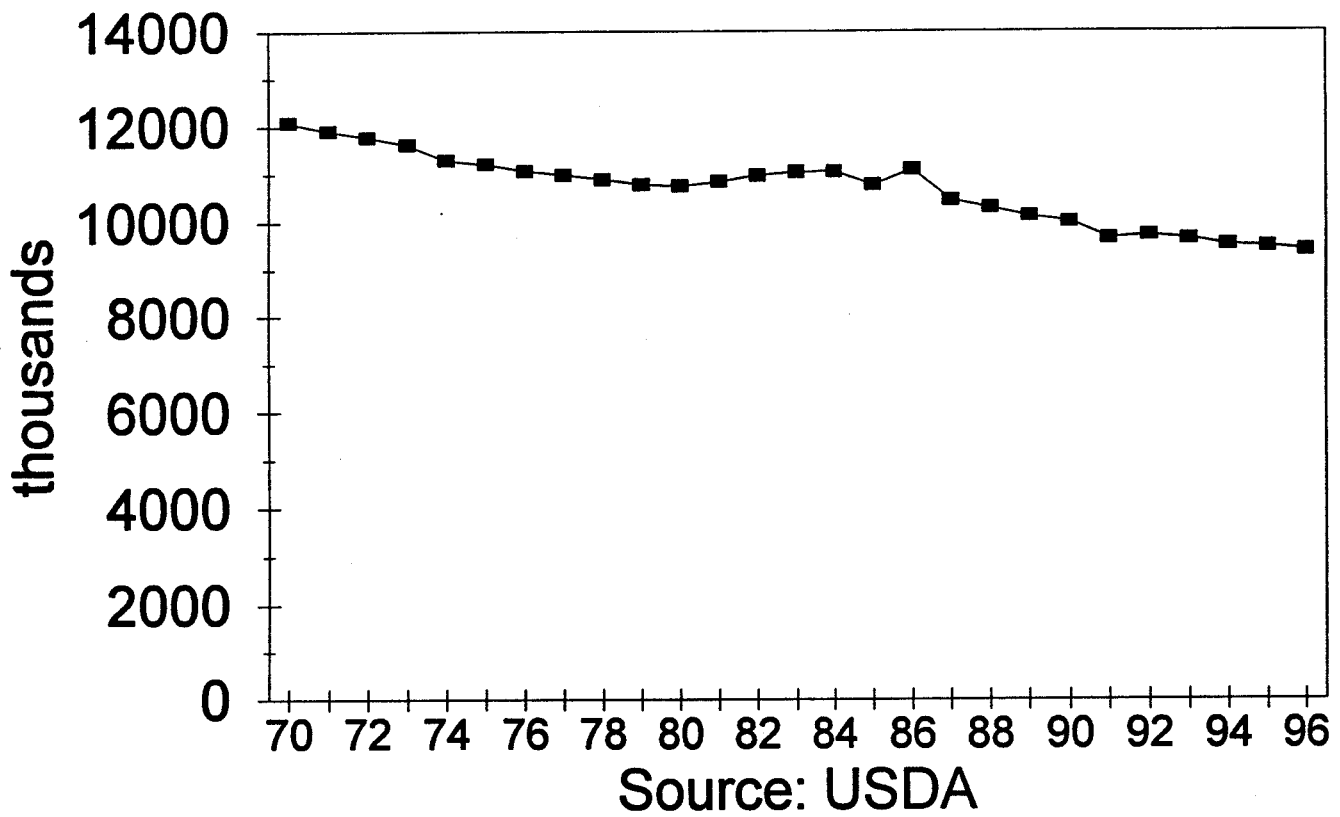
Source: USDA

Total milk production in U.S. 1970-1995

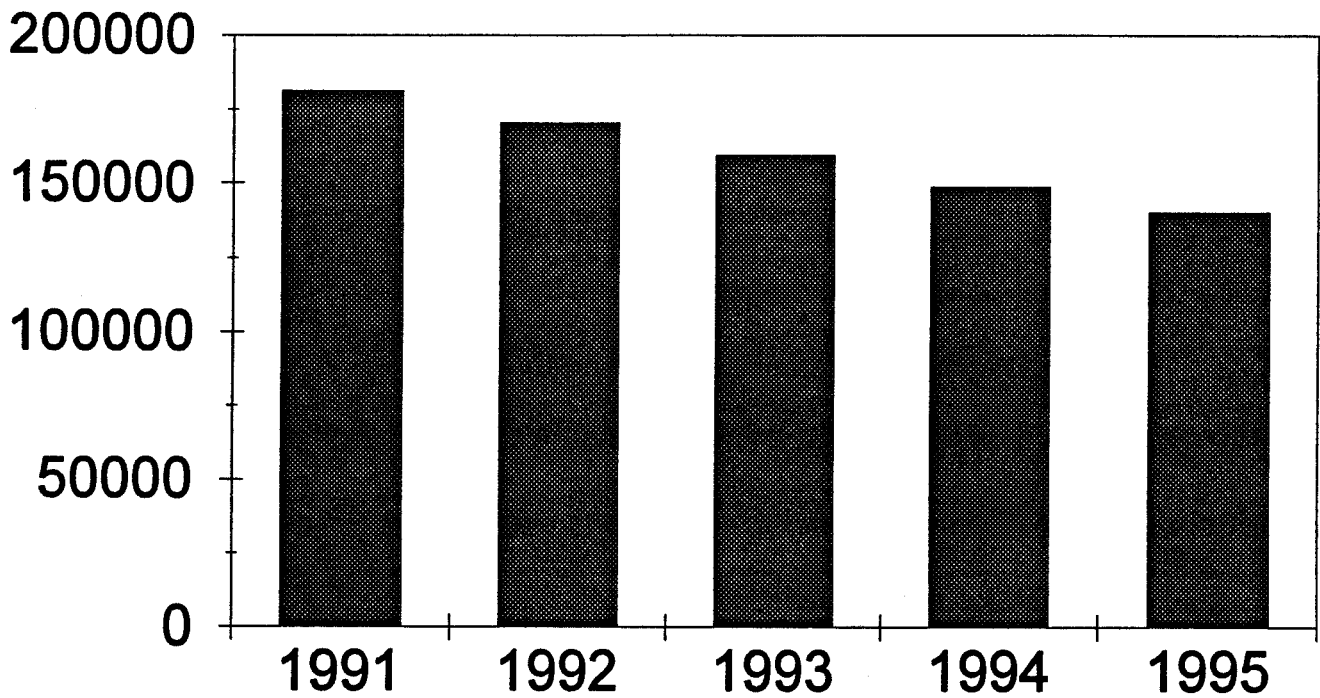


Source: USDA

Number of milk cows in the U.S. 1 January 1970-1996

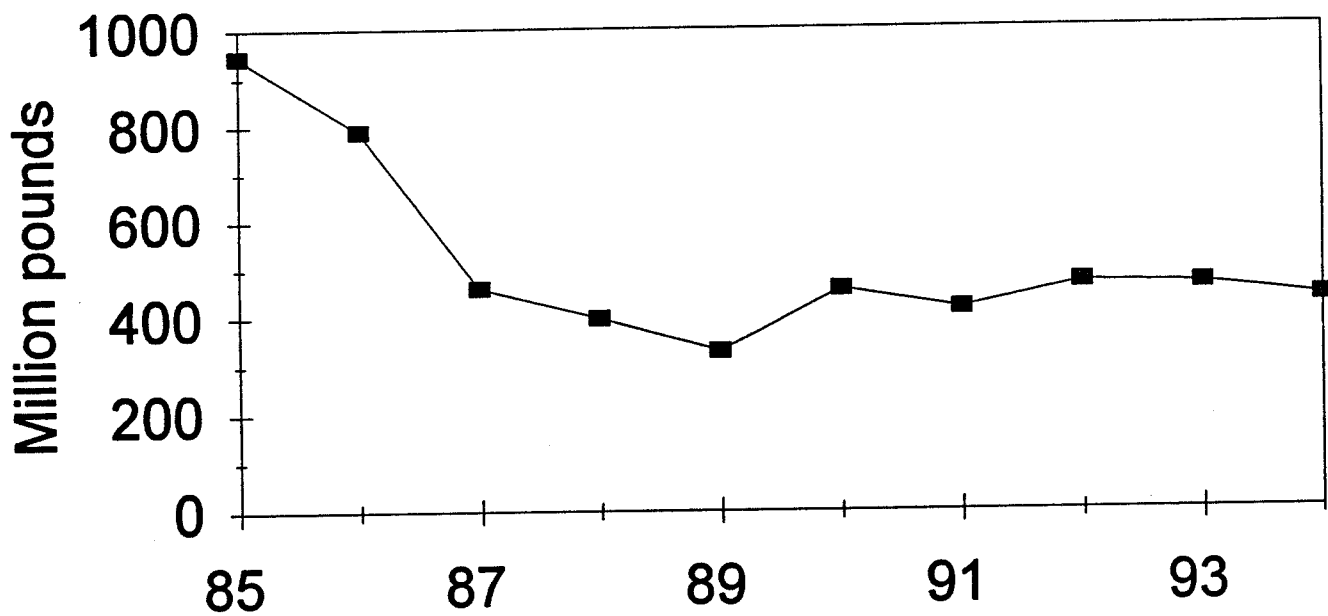


Number of dairy operations in U.S. 1991-1995



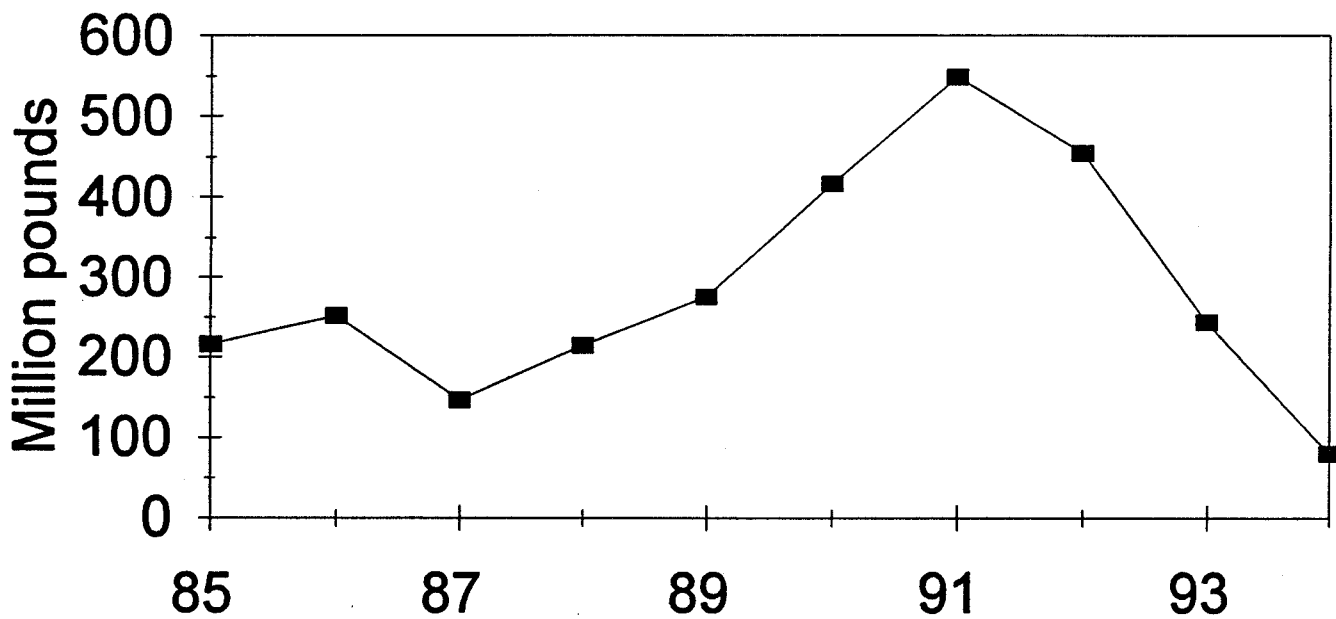
Source: USDA

Cheese stocks on hand 31 December 1985-94



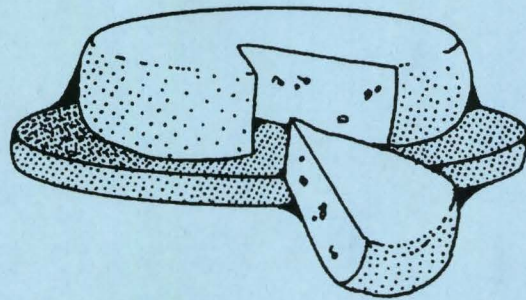
Source: USDA

Butter stocks on hand 31 December 1985-94



Source: USDA

**INNOVATION WITHIN THE
STANDARD OF IDENTITY**



**RICHARD MERRILL
LEPRINO FOODS**

Utah State University
Biennial Cheese Industry Conference

INNOVATION WITHIN THE STANDARD OF IDENTITY

BACKGROUND

On December 29, 1995, the Food and Drug Administration (FDA), in an advance notice of proposed rule making announced the agency's plans to review its regulations pertaining to identity, quality, and fill of container for standardized foods, and the common or usual name regulations for nonstandardized foods. Based on FDA's review, the agency has requested comments from all interested parties on whether these regulations should be retained, revised, or eliminated.

The driving force behind this proposed rule is the "Regulatory Reinvention Initiative" memorandum (RRI) issued by President Clinton last year. Among other things in the initiative, the President has encouraged the different government agencies and departments to work toward making Government more effective. All Americans want the benefits of effective regulation, such as wholesome food, but too often, the rules are drafted with such detailed do's and don'ts that the objectives they seek to achieve are undermined. Thus, the RRI memorandum directed departments to conduct a page-by-page review of all their regulations and eliminate or revise those that are outdated or otherwise in need of reform. With this proposed rule, the agency is announcing its intent to review the remainder of the standards of identity (in 21 CFR parts 130 through 169) and common or usual name regulations (in 21 CFR part 102).

Food standards and common or usual name regulations were enacted before the Nutrition Labeling and Education Act of 1990 and, thus, were developed without reference to the significant informational function that the food label can play. Intended to protect the integrity of the food supply, some regulations are extremely detailed and have the potential to limit technological advances. Therefore, the food standards and common or usual name regulations which cover approximately 260 pages in the Code of Federal Regulations, appear to be exactly the kind of regulations that need reform. Standards of identity and common or usual name regulations differ in their scope as follows:

A standard of identity essentially provides a "recipe" for a given food in addition to specifying the food's nomenclature. Federal standards of identity also have full preemptive authority over state standards. A common or usual name merely identifies or describes the basic nature of the food or its properties or ingredients. A common or usual name does not provide as complete a characterization of the product's composition as a standard of identity does. The scope of preemptive authority is not clearly defined with common or usual names. Common or usual name regulations also require the labeling of the percentage of any characterizing ingredient when the amount of such ingredient has a material bearing on the price or on consumer acceptance. However, the percentage labeling of characterizing ingredients may also occur with standardized food without affecting the food's standardized status.

Prior to the passage of the Nutrition Labeling and Education Act (NLEA), FDA resisted the idea of using a descriptive term with a standardized product. The original product involved with this debate was tomato juice with vitamin C added. It was finally determined that the product would be "legal, but nonstandardized." With the passage of NLEA and development of defined nutrient content descriptors, FDA developed the "generic standard" to allow the use of a nutrient content descriptor with standardized foods. In addition, the development of a generic standard prevented the "legal, but nonstandardized" status from being applied to all standardized products using a nutrient content descriptor. Therefore, in the spectrum of foods standards, standards of identity provide the strongest form of preemption. The scope of their preemptive authority is clearly defined. At the other end of the spectrum of foods standards are the common or usual name regulations where it is unclear what preemptive authority exists.

HISTORY

Food standards have now been in place for 90 years. The first law enacted was the Food and Drugs Act of 1906, which established definitions for adulteration and misbranding. However, since the 1906 act did not require foods to carry an ingredient statement on the label, the act left Government without means of comparing foods to determine occurrences of dilution or substitution. By reason of its so called "distinctive term proviso", misbranding provisions of the 1906 act actually contributed to the proliferation of cheap or adulterated foods that could be sold legally. This provision permitted the marketing of foods under the name of the food they purported to be by allowing their sale under meaningless "distinctive" names. Without the ability to establish mandatory standards under the 1906 act Government was handicapped in its attempts to maintain the food supply's wholesomeness. Congress sought to correct this deficiency in the 1906 Food and Drug Act by enacting section 401 of the Federal Food, Drug, and Cosmetic Act, which effectively provides for standards of identity, quality, and fill of container.

Misbranding provisions of the 1938 act require that foods purporting to be, or represented as, the standardized food comply with the compositional requirements of the applicable standard and bear the name designated in the definition and standard for the food. The 1938 act gave Government the authority to establish standards. Early standards of identity were primarily "recipe standards", defining both mandatory and optional ingredients to be used, and, in many instances, the procedure to be followed in manufacturing the food.

FDA has implemented section 401 of the act by adopting over 280 standards of identity. Standards may specify minimum levels of valuable constituents and maximum levels for fillers and water. They may also designate the manufacturing process required to achieve the standardized product. Standards of identity for some cheeses in part 133, specify the manufacturing process, in addition to establishing minimum milkfat and maximum moisture requirements, to distinguish one cheese from another.

DEVELOPMENTS AFFECTING THE FOOD STANDARD REGULATIONS

- Adoption of the 1958 Food Additives Amendment and the Color Additives Amendment shortly thereafter instituted a much broader, more in-depth procedure for preclearance of substances used in food, so that food safety issues largely disappeared from consideration in the adoption and amendment of standards. These amendments allowed FDA to develop its "safe and suitable" policy. Under this policy, ingredients used in food must be listed food or color additives, generally recognized as safe (GRAS) substances, and used at levels no higher than necessary to accomplish their intended functional effect in the food. This provision also requires that each safe and suitable optional ingredient used in the food be declared on the label. However, a few of the standards; cheese's being a prime example, have not been updated to increase the flexibility in the manufacture of these foods.
- The 1990 amendments amended ingredient labeling so that mandatory full ingredient labeling is required of all food products whether standardized or nonstandardized. With the assurance that all ingredients will be fully disclosed on the label, in the descending order of predominance, there is considerably less justification for a standard to limit or prescribe all ingredients that may or may not be used in the product.
- The 1990 amendment removed most requirements in which formal rulemaking is required to effect change in the standards. The only exception is for actions to amend or repeal standards of identity for dairy products.
- The 1990 amendments also require that virtually all foods bear nutrition labeling. This information, plus the full disclosure of ingredients that is now required, ensure that vastly more information about the make-up of a food is available to consumers now than was available in 1938.
- The 1990 amendments authorize FDA to adopt defined nutrient content claim regulations, such as "reduced fat." Having uniform definitions for nutrient content descriptors, the agency was able to establish a general definition and standard of identity in 21 CFR 130.10, permitting modification of traditional foods to achieve a nutrition goal, such as a reduction in fat or calories. This general definition and standard of identity requires that the modified

food: (1) not be nutritionally inferior, (2) possess similar performance characteristics as the reference food, (3) contain a significant amount of mandatory ingredients, (4) not contain an ingredient that is prohibited in the traditional standardized food. However, under 130.10, safe and suitable ingredients not specified in the standard of the traditional food can be added to ensure the modified food will not be inferior. This one standard (130.10) has awarded tremendous flexibility to manufacturers to produce foods that deviate from traditional standards. Under the general standard in 103.10, manufacturers are able to meet consumer demands for reduced fat dairy products.

ADVANTAGES AND DISADVANTAGES OF FEDERAL STANDARDS OF IDENTITY

Advantages

1. Standards protect consumers from economic fraud and promote honesty and fair dealing in the interest of consumers.
2. Standards provide assurance to consumers of product uniformity, with the resulting expectation and belief by consumers that all products bearing a particular name will possess the same characteristics irrespective of where they are purchased, or by whom they are manufactured or distributed.
3. Standards are an efficient mechanism for addressing public health problems through mandatory fortification requirements.
4. Standards provide manufacturers with guidance in the production, naming, and labeling of products and with the assurance that competitors will have to meet the same guidelines for the same foods.
5. Standards promote consistency in labeling and serve as a basis for nutrient content claims.
6. U.S. food standards reflect, for the most part, current commercial practices in this country and serve as a useful basis for negotiations in determining international standards.
7. Federal standards of identity promote uniformity by preempting state standards (added by IDFA).

Disadvantages

1. Standards may serve as an impediment to the food industry to the degree to which they fail to reflect advances in food science and technology.
2. Incorporation of advances in food technology may be difficult or impossible without laborious amendment of the relevant standard; however, nonstandardized foods are able to take advantage of these new technologies.

INTERNATIONAL DAIRY FOODS ASSOCIATION RESPONSE TO THE FDA ADVANCED NOTICE OF PROPOSED RULEMAKING

Elimination of Federal Preemption; Impact on State Jurisdiction

- Without minimizing the other benefits that standards of identity provide, an overriding value of federal standards is the assurance they provide that regulation of food composition and labeling, and in particular for dairy products, will be uniform throughout the nation. Certainly an important impetus for the initial adoption of dairy standards in the 1930s and 1940s, and thereafter, was the growing recognition that products widely distributed in interstate commerce should be subject to a single set of compositional and labeling requirements.

Because milk and other dairy products are produced, processed and marketed in every state of the country, and because of the very substantial significance of the dairy industry in every state, state legislators and regulatory officials frequently felt called upon to impose their own concepts of how these products might be regulated. But there is no question that the standards of identity provide a much stronger basis for the industry to advance its arguments concerning the importance of uniformity of regulation throughout the country.

It was not until the enactment of the NLEA, however, adding express federal preemption provisions in new section 403A of the FD&C Act, that state officials generally came to recognize fully their obligation to bring their regulation of dairy products into line with the standards prescribed by the FDA. these uniformity issues may not have entirely

disappeared, but they are no longer of significant concern, at least for those dairy products that are governed by one of the federal standards in Parts 130, 131 or 133. The industry may thus be seeing an end to the burdensome production, labeling and marketing costs that have been unnecessarily imposed upon the industry, and indirectly on consumers, for so many years by varying state regulatory requirements, to say nothing of the costs to states and the industry of adopting, enforcing and litigating non-uniform dairy standards.

This not-so-ancient history makes it clear that the revocation of the federal standards of identity for dairy products would lead to the immediate revival of varying state requirements, resulting in overwhelming disruption in the production, labeling and marketing of these products in interstate commerce.

Overall Operation of Food Standards

1. Utility of the System

- FDA raises the question of whether standards should be partially or totally eliminated. IDFA would vigorously oppose any suggestion that the concept of food standards has outlived its usefulness or that standards no longer serve the public interest. The wholesome elimination of food standards, as distinguished from appropriate modification of them, would achieve little conceivable benefit to consumers, to industry, or to FDA. Where there are defects in standards, they may be remedied through modification, consolidation and, in some cases, revocation (as in the case of lower fat dairy product standards). But in no sense does the existence of some outdated, unduly restrictive provisions in standards provide support for the view that the concept of standards themselves is no longer viable.
- FDA request comments on whether consumers find the current system of standards meaningful. IDFA has no information as to whether consumers find food standards meaningful. Rather, IDFA is satisfied that consumers in general have no awareness of the concept of standards of identity. They are interested in the information provided on the label, and the characteristics of various products that appeal to their taste, preferences and uses. Standards serve those interests, even though most consumers are unaware of the regulatory mechanism at work.
- FDA requests whether industry needs compositional food standards for orderly marketing of foods, with particular reference to cheeses and ice cream. Standards are of value for establishing the basic benchmarks of product identity and fundamental characteristics. They are extremely useful, and provide significant consumer benefits, to the extent that they define a finished product so that consumer expectations as to the nature of the food will not be disappointed. Thereafter, of course, product variation is in the hands of the processor, and consumer preferences and marketing decisions will ultimately decide what is made and sold. Certainly the compositional requirements set forth in many dairy standards provide a useful basis for identifying the characteristics of a food that are so closely identified with its identity.

International Standards

- IDFA is in full agreement that in the absence of federal food standards, the position of the United States delegates at Codex Committee meetings would be significantly weakened. In addition, to the extent that U.S. standards and Codex standards can be harmonized, or at least more so than they are now, international trade between the United States and the rest of the world in food products will be greatly facilitated.

Concerns have also been expressed by a number of IDFA members that the United States Government has not evidenced a sufficiently strong commitment to this country's participation in the Codex process. Perhaps driven by the view that U.S. standards will never be overtaken by Codex standards, U.S. representatives have been less aggressive, and less effective, in dealing with their foreign counterparts in the standards establishment process. Accordingly, IDFA favors reasonable efforts to harmonize international and U.S. standards, from both sides, by modification of U.S. standards when appropriate, and through a stronger commitment to U.S. participation in the international standards-setting process.

Economic Issues

- FDA requested comments on the extent to which standards of identity may produce confusion rather than provide important information to consumers. The experience of the dairy industry is that rigid standards may indeed become misleading or confusing over time, as the factual predicates upon which the original standards were based themselves change or become less important. To the extent that standards can be made more flexible, while retaining important benchmarks, it will be far easier for companies to respond to changing circumstances and conditions by reformulating standardized products without amendment of the regulations.
- FDA requests comments on the proper degree of flexibility for particular standards. As a general rule, IDFA and its members believe that a reasonable degree of flexibility is desirable, but that that issue can be resolved meaningfully only in the context of particular standards and the products they cover. Standards serve best when they serve the basic purposes of prescribing a benchmark of certain minimum characteristics that are closely associated with the identity of the product. Significant flexibility beyond that is desirable, except to the extent that such flexibility might be exploited in individual circumstances to enable departure from those fundamental benchmarks. For example, in certain standards a very broad safe and suitable ingredient provision may be desirable. In other standards, it may be necessary to limit options that might be seen as undercutting the benchmark provisions of the standards.
- FDA suggests that federal standards may not be superior to state standards because they may have different costs and benefits associated with them. These statements by FDA misperceive the proper relationship between federal and state standards, and the benefits they might provide. If there are provisions that were contained in formerly effective state standards, or proposed new provisions for state standards that might be more beneficial than federal standards, then the proper forum for consideration of those changes is at the federal level so that the uniform national standard itself can benefit from any asserted improvement that such change might yield. FDA makes no effort to support its suggestion that the benefits provided by a particular varying state standard might exceed the costs that would be visited upon the industry, and thereby on consumer, resulting from varying state labeling and compositional standards.

Common or Usual name regulations

- The ANPR questions whether there is a need to continue maintenance of the common or usual name regulation procedures. None of those regulations have been adopted for dairy products, and IDFA has no position on whether the existing common or usual name regulations should be maintained or discontinued. In view of the fact that NLEA now authorizes the use of notice and comment procedures for the adoption of new standards of identity, and both FDA and industry recognize that standards of identity are simpler and less prescriptive than common or usual names, a separate common or usual name approach is no longer necessary or desirable for a new standard.

Declaration of Percentage of All Major Ingredients

- IDFA regards this as a wholly unsatisfactory substitute for the foods standards program. In a few limited circumstances, a declaration of the percentage of a particular ingredient may appear to be an appropriate choice. For the most part, however, IDFA believes that declaration of percentages provides no meaningful information to consumers. The compositional requirements or benchmarks of the dairy standards assure that consumer expectations will not be disappointed when they purchase a product with a particular statement of identity. As FDA comments point out, declaration of percentages would also severely stifle the ability of companies to make minor changes in product formulations, entirely consistent with the benchmark compositional requirements of the standards.

Agency Budget Constraints

- IDFA opposes in the strongest possible terms any proposal for FDA to seek fees to support its standards program. Food standards are not individual company licenses or proprietary franchises. They serve clearly identifiable consumer interests and are part of FDA's basic mission to prevent consumer confusion and deception.

Concluding Remarks

- These comments have documented the dairy industry's commitment to the FDA food standards program. Modification and simplification are certainly called for, but not elimination. In 1990 Congress reaffirmed the value of uniform national standards when it enacted section 403A and simplified the procedures for implementing section 401. FDA has no reasonable option other than to implement those decisions.

NATIONAL CHEESE INSTITUTE STANDARDS TASK FORCE

Some have suggested to get rid of food standards and allow market forces to control the composition of the products that are currently regulated by standards. Thoughts of eliminating cheese standards were entertained by the task force, though only for moments. It quickly became clear that eliminating cheese standards would return the cheese industry to the past when states instituted their own standards. Cheese products are produced and marketed in every state of the country and without minimizing the other benefits that cheese standards provide, an overriding value is the preemption provision enacted in section 403A. In the absence of cheese standards states would be free to impose their own concepts of how cheese products should be regulated.

Certainly dairy standards of identity are in need of reform. Elimination of nutrient content-related standards is appropriate. The agency has already proposed to revoke the current standards for lower fat versions of milk, sour cream (sour half and half), cottage cheese and yogurt products. The intent of this action is to have these nutrient-modified products covered by the use of the generic standard (21 CFR 130.10) and the standard of identity for the parent product. With the proposed FDA rule, the NCI intends to take this opportunity to eliminate and revise (table 1) as many cheese standards as is appropriate.

Of the 280 standards of identity adopted by FDA over 25% (73 standards for cheese) are specifically cheese standards. Of the 73 cheese standards, two cheeses could be proposed for immediate elimination based solely on the fact that they carry nutrient content descriptors (e.g., low sodium cheddar and colby) and should be regulated through NLEA and the parent standard. In addition, part skim cheeses should be considered for elimination or revision to allow NLEA regulations to govern labeling of these products. In addition to eliminating standards many standards will be concurred for collapse into one standard. For example process cheese could be regulated by a general standard that would take into account all of the various varieties of process cheese. There also exist many standards that the NCI task force have no information about, such standards will be investigated to determine if the variety is being produced. In the case where standards exist but are no longer being produced elimination of these obsolete standards would be proposed.

The NCI goals for reforming cheese standards are as follows:

1. Cheese standards will continue to promote uniformity by preempting state standards.
2. Cheese standards, where appropriate will be changed to allow greater flexibility in the use of safe and suitable ingredients.
3. Cheese standards will continue to protect consumers from economic fraud and provide assurance of product uniformity.
4. Cheese standards, where appropriate will eliminate or revise defined manufacturing methods to allow greater flexibility to manufacturers of these products.
5. Glean the advantages from Codex standards, moving as close to Codex harmonization as possible.

Included for your review are draft copies of a general cheese standard and a draft of the mozzarella cheese standard. Also included are drafts of Canada's and New Zealand's proposed cheese standards formats. The task force has basically agreed to the format of the general cheese standard as shown. However, with the individual cheese variety standards the task force is still reviewing format proposals. A table format similar to the Canadian's is being considered. Although, questions have been raised as to whether or not a table format can adequately capture all the information need to make standards effective.

It is the NCI task forces intention to submit all of the proposed changes to FDA as a single proposal, rather than submit each standard individually. In all likelihood it should be possible to reduce the number of cheese standards of identity by half. If the task force can agree to a format it should be possible to have the proposal for natural cheese to FDA sometime in mid-1997.

The opportunity to revise cheese standards that have been in place for over thirty years is one that the task force looks forward to. The task force also realizes the responsibility it has to ensure that through this process, cheese products are not cheapened or debased. Members of the task force look see new, more flexible standards with open eyes to the future of our industry. We believe that through revision of the standards that regulate our industry, that the dairy industry will be better equipped to meet consumers changing tastes. New cheese standards will better prepare the U.S. dairy industry for the global marketplace, better preparing our companies to compete internationally, and against other food products domestically.

Table 1: SUMMARY OF SUGGESTED ACTIONS ON CHEESE STANDARDS

	PRODUCT	SUGGESTED ACTION	
133.102	Asiago fresh and asiago soft cheese	Collapse into one standard	
133.103	Asiago medium cheese		
133.104	Asiago old cheese		
133.106	Blue cheese	Keep	
133.108	Brick cheese	Keep	
133.109	Brick cheese for manufacturing	Create Generic standard for manufacturing	
133.111	Caciocavallo siciliano cheese	Investigate	
133.113	Cheddar cheese	Keep	
133.114	Cheddar cheese for manufacturing	Create Generic standard for manufacturing	
133.116	Low sodium cheddar cheese	Eliminate (NLEA Claim)	
133.118	Colby cheese	Keep	
133.119	Colby cheese for manufacturing	Create Generic standard for manufacturing	
113.121	Low sodium colby cheese	Eliminate (NLEA Claim)	
133.123	Cold-pack and club cheese	Collapse into one standard (at least collapse cheese foods standards)	
133.124	Cold-pack cheese food		
133.125	Cold-pack cheese food with fruits, vegetable or meats		
133.127	Cook cheese, Koch kaese	Investigate	
133.128	Cottage cheese	Collapse into one cottage cheese standard	
133.129	Dry curd cottage cheese		
133.131	Lowfat cottage cheese	Eliminate (NLEA Claim)	
133.133	Cream cheese	Collapse into one standard	
133.134	Cream cheese with other foods		
133.136	Washed curd and soaked curd	Create Generic standard for manufacturing	
133.137	Washed curd cheese for manufacturing		
133.18	Edam cheese	Keep	
133.140	Gammelost cheese	Investigate	
133.141	Gorgonzola cheese	Keep	
133.142	Gouda cheese	Keep	
133.144	Granular and stirred curd cheese	Create Generic standard for manufacturing	
133.145	Granular cheese for manufacturing		
133.146	Grated cheese	Class standard	
133.147	Grated American Cheese Food	Generic standard	
133.148	Hard grating cheese	Keep	
133.149	Gruyere cheese	Keep	
133.150	Hard cheeses	Class standard	
133.152	Limburger cheese	Keep	
133.153	Monterey cheese and Monterey jack cheese	Collapse into one standard	
133.154	High-moisture jack cheese		
133.155	Mozzarella cheese and scamorza cheese	Collapse into one standard	
133.156	Low-moisture mozzarella and scamorza cheese		
133.157	Part-skim mozzarella and scamorza cheese		
133.158	Low-moisture part-skim mozzarella and scamorza cheese		
133.160	Muenster and munster cheese	Keep	
133.161	Muenster and munster cheese for manufacturing	Create Generic standard for manufacturing	
133.162	Neufchatel cheese	Investigate incorporation into cream cheese standard	
133.164	Nuworld cheese	Incorporate into another standard	
133.165	Parmesan and reggiano cheese	Keep	
133.167	Pasteurized blended cheese	Collapse into one process cheese standard	
133.168	Pasteurized blended cheese with fruits, vegetables, or meats		
133.169	Pasteurized process cheese		
133.170	Pasteurized process cheese with fruits, vegetables, or meats		
133.171	Pasteurized process pimento cheese		
133.173	Pasteurized process cheese food		
133.174	Pasteurized process cheese food with fruit, vegetables, or meats		
133.175	Pasteurized cheese spread		
133.176	Pasteurized cheese spread with fruit, vegetables, or meats		
133.178	Pasteurized Neufchatel cheese spread with other foods		Problematic - possibly incorporate into neufchatel standard
133.179	Pasteurized process cheese spread		Collapse into process cheese standard
133.180	Pasteurized process cheese spread with fruit, vegetables, or meats		
133.181	Provolone cheese	Keep	
133.182	Soft ripened cheese	Keep	
133.183	Romano cheese	Keep	
133.184	Roquefort cheese, sheep's milk blue-mold, and blue-mold cheese from sheep's milk	Consider a blue-veined cheese standard and specify differences	
133.185	Samsoe cheese	Investigate	
133.186	Sap sago cheese	Investigate	
133.187	Semisoft cheese	Class standard	
133.188	Semisoft part-skim cheeses	Class standard	
133.189	Skim milk cheese for manufacturing	Investigate (process cheese manufacturers)	
133.190	Spiced cheeses	Collapse into one standard	
133.191	Part-skim spiced cheese		
133.193	Spiced, flavored, standardized cheeses		
133.195	Swiss and Emmentaler cheese	Keep	
133.196	Swiss cheese for manufacturing	Create Generic standard for manufacturing	

** Consider creating a generic standard for cheese with fruits, vegetables, and meats

** Consider creating a standard for ricotta cheese

PROPOSED CFR GENERAL CHEESE STANDARD

1. SCOPE

This Standard applies to all products in conformity with the definition of cheese in paragraph 2 of this standard and intended for direct consumption or further processing, including those varieties of cheese for which individual or group standards have been elaborated. Standards for individual varieties of cheese, or groups of varieties of cheese may contain provisions which are more specific than those in this standard and in such cases those more specific provisions shall apply to the individual variety of groups of varieties of cheese.

In addition, subject to the provisions of this standard, and the provisions of the applicable individual standard, any cheese modified to meet the definition of a nutrient content claim shall be subject to the provisions of 21 CFR 130.10 *Requirements for foods named by use of a nutrient content claim and a standardized term.*

2 DESCRIPTION

2.1 Definitions

Cheese is the cured or uncured solid or semi-solid product obtained by:

- (a) coagulating the following raw materials:] milk (as defined in CFR 130.3), nonfat milk (as defined in 21 CFR 130.3), cream (as defined in 21 CFR 130.3), whey cream, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation; or
- (b) processing techniques involving coagulation of milk and/or materials obtained from milk which give an end-product which have the same physical, chemical and organoleptic characteristics as the product defined under (a).

2.1.1 Cured or ripened cheese is cheese which is not ready for consumption shortly after manufacture but which must be held for such a time, at such a temperature, and such other conditions as will result in the necessary biochemical and physical changes characterizing the cheese.

2.1.2 Mold cured or mold ripened cheese is a cured cheese in which the curing has been accomplished primarily by the development of characteristic mold growth throughout the interior and/or on the surface of the cheese.

2.1.3 Uncured, unripened, [including fresh cheese] is cheese which is ready for consumption shortly after manufacture.

3. COMPOSITION FACTORS

3.1 Dairy ingredients

-Only those raw materials indicated in the definition of cheese in 2.1 are permitted and in addition:

3.2 Permitted ingredients

Safe and suitable ingredients, except vegetable fat and oils, including flavoring materials, may be added, provided that the ingredient is not intended to take the place of

any milk constituent, unless the cheese has been produced according to the provisions of 21 CFR 130.10.

4 LABELING

The products covered by this Standard shall be labeled in accordance with 21 CFR Part 101 and 21 CFR 130.10.

4.1 Name of the food

Only products in conformity with this standard may be designated cheese. This applies to products for which no individual varietal standard exists and/or products manufactured under the provisions of 21 CFR 130.10.

4.1.1 In the absence of a standardized product the name "cheese" shall be accompanied by the appropriate designation in accordance with the classification of cheese in the following table.

Note to Task Force: this section need additional thought. The concept exists in both the Codex and IDF standards and may be useful. However, the concept of "moisture on a fat-free basis" is not widely used in the U.S.. Can "total moisture" be employed?

(???????)		Term I	Term II
If the moisture is %	If the MFFB* is %	The 1st phrase in the designation shall be	Designation according to principal curing characteristics
<42	<51	Extra hard	1. Cured or ripened
43-46	52-56	Hard	a. mainly surface
47-50	57-63	Semi-hard	b. mainly interior
51-56	64-69	Semi-soft	2. Mold cured or ripened
>56	>70	Soft	a. mainly surface
			b. mainly interior
			3. Uncured or unripened

*MFFB equals percentage moisture on a fat-free basis, i.e.

$$\frac{\text{Weight of moisture in the cheese}}{\text{Total weight of cheese} - \text{Weight of fat in the cheese}} \times 100$$

Example:

The description of a cheese with a moisture of 48 and a moisture on a fat-free basis of 57% of which is cured in a manner similar to that in which Roquefort is cured would be:

Semi-hard Interior mold cured cheese
(Term I) (Term II)

4.1.2 Where milk, other than the type of milk traditionally used, is used for the manufacture of the product, a word or word denoting the animal from which the milk has been obtained shall be inserted immediately before or after the designation of the product, and where milk from more than one species of animal is blended the milk from the different species shall be declared in descending order of proportion calculated on the basis of dry matter. Such declarations are not required if the consumer would not be misled by their omission or where the origin of milk is specified in individual or group standards.

PROPOSED NEW STANDARD - MOZZARELLA [and SCAMORZA] CHEESE

1. SCOPE

This Standard applies to Mozzarella [and Scamorza] cheese intended for direct consumption or for further processing in conformity with the description in para 2 of this standard. The name of the product may be used exclusively for cheese complying with this standard.

2. DESCRIPTION

Mozzarella [*and Scamorza*] cheese is an unripened soft cheese in conformity with the General Cheese Standard, and prepared from dairy and other ingredients by the "pasta filata" procedure set forth in this section or other processing techniques, which produce a finished cheese having the same organoleptic, physical, and chemical properties set forth in this paragraph.

The body has a fibrous texture and is typically rindless. It is ready for consumption after manufacture. The cheese may be formed into many shapes, or may be shredded or sliced. The cheese is mainly stored under refrigeration under which it has limited shelf-life, but may also be stored frozen.

2.1 Definitions

Pasta filata processing consists of heating curd of a pH value suitable for further processing and kneading and stretching until smooth and free from lumps. Still warm the cheese is shaped and formed, then firmed by cooling.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 Permitted ingredients

Cheese shall be produced from cow's or buffalo milk (or their mixtures) and products obtained from those milks, and any safe and suitable [non-dairy] [functional] ingredient which give an end-product similar in physical, chemical and organoleptic characteristics to those discussed in paragraph 2 and is in conformity with paragraph 3.2 of this standard, unless the cheese has been produced according to the provisions of 21 CFR 130.10.

3.2 Composition Designation

		Mozzarella
Total [Wet] Fat	min%	18
Moisture	min%	45
	max%	60

4. LABELING

4.1 Name of the Food

Only products in conformity with this standard may be designated Mozzarella [or Scamorza]. Should buffalo milk be used, totally or in mixture with cow's milk, for the manufacture of the product, the designation will include a reference to the type(s) of milk used.

DRAFT

Part 133 - CHEESES AND RELATED CHEESE PRODUCTS

Subpart A - General Provisions

133.3 Definitions

- (a) *Milk*
- (b) *Nonfat milk*
- (c) *Cream*
- (d) *Pasteurized*
- (e) *Ultrapasteurized*
- (f) *Ripened* cheese is cheese which is not ready for consumption shortly after manufacture but which must be held for such a time, at such a temperature, and such other conditions as will result in the necessary biochemical and physical changes characterizing the cheese in question.
- (g) *Surface ripened* cheese is cheese in which the ripening process begins on the surface of the cheese and develops further into the body of the cheese.
- (h) *Mold ripened* cheese is a ripened cheese in which the ripening process has been accomplished primarily by the development of characteristic mild growth throughout the interior and/or on the surface of the cheese.
- (i) *Unripened* cheese including fresh cheese is cheese which is ready for consumption shortly after manufacture.

133.100 Cheese

- (a) *Scope.* this general standard applies to all products conforming to the description of cheese in (b) of this section and intended for direct consumption or further processing, including those varieties of cheese for which individual varietal or group varietal standards have been established. Standards for individual varieties of cheese, or groups of varieties of cheese may contain provisions which are different from those in this standard. Such provisions shall apply only to the individual varietal or group standard in which the differing provisions are cited.

In addition, subject to the provisions of this standard, and the provisions of the applicable individual or group varietal standard, any cheese modified to meet the definition of a nutrient content claim shall be subject to the provisions of 21 CFR 130.10 *Requirements for foods named by use of a nutrient content claim and a standardized term.*

- (b) *Description.* Cheese is the cured or uncured solid or semi-solid product prepared by:
 - (1) coagulating milk, nonfat milk, cream, as defined in 133.3, or any combination of these materials through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation; or
 - (2) processing techniques involving coagulation of milk and/or safe and suitable ingredient derived from milk which give an end product which has essentially the same physical, chemical, and organoleptic characteristics as the product defined in (1).
- (c) *Optional ingredients.* The following safe and suitable ingredients may be used:
 - (1) *Dairy ingredients.* Milk, nonfat milk, cream, as defined in 133.3, or other safe and suitable ingredients derived from milk, the use of milk and milk products from other milk source animals (e.g. goat, sheep, water buffalo) is permitted.
 - (2) *Nondairy ingredients.* Safe and suitable ingredients including, but not limited to, bacterial cultures, enzymes, colors, antimicrobials, anti-caking agents, firming agents, and flavoring materials. [option 1 - Such ingredients may be used to assist in processing, improve

texture, add flavor, prevent syneresis, extend shelf life, or improve appearance, but shall not be used to replace essential dairy ingredients (e.g., vegetable oil may not be used to replace milkfat).] or [option 2 - Cheese shall contain no foods fats or proteins other than those derived from milk, except fat or proteins which may be natural components of flavoring ingredients, or fats and proteins which are added in incidental amounts to accomplish specific functions.]

- (d) Nomenclature. The name of the food is "cheese" or "_____cheese" (the blank being filled in with the name of the individual or group varietal standard, if applicable). When the food is made with milk or milk products from milk source animals other than cows, the name of the food is accompanied by the phrase "made with _____milk" (the blank being filled in with the name(s) of all source milk animals). Descriptive terms indicating ripening characteristics (as described in 133.3) and/or firmness (as described in this section) may accompany the name of the food as appropriate:

Descriptive Term - Firmness	Moisture Requirement - Fat Free Basis *
Extra hard	less than 51% moisture
Hard	not less than 51%, but less than 57% moisture
Firm or Semi-hard	not less than 57%, but less than 63% moisture
Semi-soft	not less than 63%, but less than 70% moisture
Soft	70% or more moisture

* Percentage moisture on a fat free basis is determined as follows:

$$\frac{\text{Weight of moisture in the cheese}}{\text{Total weight of cheese} - \text{Weight of fat in the cheese}} \times 100$$

- (e) Label declaration.
- (1) enzymes of animal, plant, or microbial origin may be declared as "enzymes"
 - (2) The dairy ingredients may be declared, in descending order of predominance, by the use of the terms "milkfat and nonfat milk" or "nonfat milk and milkfat", as appropriate.
 - (3) Declaration of dairy ingredients derived from milk source animals other than cows shall include the name of the source milk animal.

DRAFT

133.155 Mozzarella cheese

- (a) *Scope.* this standard applies to the product conforming to the description in (b) of this section. Cheese produced according to the provisions of this standard shall also conform to the provisions of the general standard found in 133.100. In addition, mozzarella cheese which has been modified to meet the definition of a nutrient content claim shall be subject to the provisions of 130.10 *Requirements for foods named by use of a nutrient content claim and a standardized term.*
- (b) *Description.* Mozzarella cheese is the food prepared from:
 - (1) milk, nonfat milk, or cream, or any combination of these materials by the process described in *Appendix 1* of this section; or
 - (2) milk and/or safe and suitable materials derived from milk by processing techniques which give and end product which has essentially the same physical, chemical, and organoleptic characteristics as the product defined in (1).
- (c) *Composition*
The minimum milkfat content is 18 percent by weight of the food. the moisture content is more than 45 percent, but not more than 60 percent by weight of the food. the protein content shall be not less than 20 percent by weight of the food. The protein to meet the minimum protein requirement shall be provided by nonfat milk solids and/or other milk-derived ingredients.
- (d) *Nomenclature.* The name of the food is "mozzarella cheese".

Appendix 1 - Traditional Manufacturing Process

Milk, nonfat milk, or cream or any combination of these materials is warmed and subjected to the action of a lactic acid-producing bacterial culture. One or more clotting enzymes are added to set the dairy ingredients to a semisolid mass. the whey is drained from the semisolid mass, and the resulting curd is ripened to develop a pH suitable for further processing. the curd is then heated and is kneaded and stretched until smooth and free from lumps. The cheese is shaped and formed, then firmed by cooling.

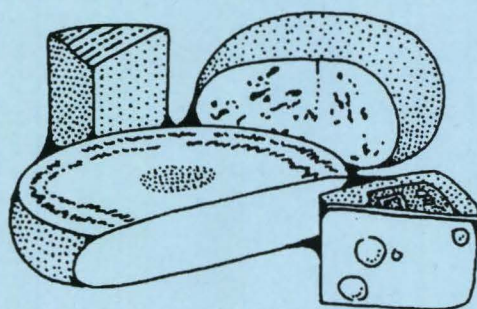
PROPOSED NEW CANADIAN STANDARDS FOR CHEESE

Item	Variety of Cheese	Column II Maximum % of moisture	Column III Minimum % of milk fat
1	Asiago	40	30
2	Baby Edam	47	21
3	Baby Gouda	45	26
4	Blue	47	27
5	Butter (Butterkase)	46	27
6	Bra	36	26
7	Brick	42	29
8	Brie	54	23
9	Caciocavallo	45	24
10	Camembert	56	22
11	Canadian Style Brick	42	29
12	Canadian Style Munster	46	27
13	Cheddar	39	31
14	Colby	42	29
15	Danbo	46	25
16	Edam	46	22
17	Elbo	46	25
18	Emmenthaler	40	27
19	Esrom	50	23
20	Farmer's	44	27
21	Feta	55	22
22	Fontina	46	27
23	Fynbo	46	25
24	Gouda	43	28
25	Gournay	55	33
26	Gruyere	38	28
27	Havarti	50	23
28	Jack	50	25
29	Kassert	44	27
30	Limburger	50	25
31	Maribo	43	26
32	Montasio	40	28
33	Monterey	44	28
34	Mozzarella (Scamorza)	52	20
35	Muenster (Munster)	50	25
36	Neufchatel	60	20
37	Parmesan	32	22
38	Part Skim Mozzarella	52	15
39	Part Skim Pizza (Delete)	48	15
40	Pizza	48	20
41	Provolone	45	24
42	Romano (Sardo)	34	25
43	St. Jorge	40	27
44	Saint-Paulin	50	25
45	Samsoe	44	26
46	Tilsiter	45	25
47	Tybo	46	25

DRAFT NEW ZEALAND PROPOSAL

Number	Title	Ripening	Texture	Other permitted ingredients	Reference standard to define characteristics	Min FDM %	Min DM %	Name of food	Permitted additives
C-1	Cheddar	Ripened	Hard	Safe and suitable enzymes to assist flavor development		48			Additive to be specified by class as far as possible
C-5	Gouda	Ripened	Semi-hard			48	57	Gouda	
						45	55	Baby Gouda	
C-8	Cheshire	Ripened	Hard	Safe and suitable enzymes to assist flavor development		48	56	Cheshire	
C-10	Gruyere	Ripened	Hard			45	62	Gruyere	
C-16	Cottage	Unripened	Soft	Gelatin		None	20	Cottage cheese	
						4	20	Creamed cottage cheese	
C-34	Brie	Mold ripened Surface ripened	Soft	Cultures of <i>Penicillium camembertii</i>		45	43	Brie or Petite Brie	
						40	42	Brie 40 or Petite Brie 40	
						50	45	Brie 50 or Petite Brie 50	
						60	40	Brie 60 or Petite Brie 60	

**CODEX ALIMENTARIUS
CHEESE STANDARDS**



**DUANE R. SPOMER
UNITED STATES DEPARTMENT OF AGRICULTURE**

CODEX ALIMENTARIUS CHEESE STANDARDS

Duane R. Spomer
United States Department of Agriculture

The term Codex Alimentarius is taken from Latin and means food code. And that's just what Codex Alimentarius is: a code of food standards for all nations.

Codex was developed as an international commission established in 1962 when two organizations, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) recognized the need for international standards to guide the world's growing food industry and to protect the health of consumers. The stated purpose of Codex Alimentarius is "... to guide and promote the elaboration and establishment of definitions and requirements for foods, to assist in their harmonization and, in doing so, to facilitate international trade."

To industrialized countries, Codex has become the ultimate reference. "What does Codex say?" is a question asked often by food technologists, manufacturers, government officials, and consumer advocates as they ponder food-related matters. To developing countries, Codex is recognized as a ready-made set of standards and guidelines. Whether adopted into law entirely or simply as reference, Codex standards provide consumer protection, and both domestic food producers and importers know that the requirements accepted in international trade.

Today there is little doubt that Codex Alimentarius has had a great impact on the quality and safety of the world's food supply. Codex has helped to upgrade standards for food manufacturing, processing, safety and quality all over the world and has contributed to an increase in international food trade since 1962. However, until the completion of the Uruguay Rounds of the General Agreements on Tariffs and Trade (GATT) and the establishment of the World Trade Organization (WTO), Codex was little known outside of those actively involved in Codex. Since then interest in the development of Codex standards has increased significantly.

In the GATT agreement, Codex standards are given a central position and are standards that will be benchmarks used in trade dispute under the WTO. These agreements have given Codex Standards the teeth they did not have before as voluntary agreements. Henceforth compliance with Codex Standards can be the key to acceptance in international trade.

To address the role of Codex standards and what are now binding obligations on governments in that organization, it is important first to understand the two different WTO agreements under which the Codex standards are relevant.

The Technical Barriers to Trade (TBT) Agreement and the Sanitary and Phytosanitary (SPS) Agreement are two very distinct agreements. Their references to Codex standards are different and they have different legal implications. The TBT Agreement covers all technical regulations (mandatory requirements), voluntary standards and conformity assessment procedures. The SPS Agreement covers any kind of measure whose purpose is to:

- protect human health from food-borne risks and animal carried diseases;
- protect animal health from risks in feedstuffs and from animal diseases and
- protect plant health.

One good example that has been used to explain the difference between TBT and SPS issues is with bottled water. If the requirement is that the bottles have to be made of a material that cannot contaminate the water, the risk and concern is with the contamination of the beverage and it is an SPS issue. If the requirement is that the bottle has to be a certain shape, a certain size, or made of a certain material then it is a TBT issue.

The SPS Agreement explicitly recognizes the right of governments to take measures to protect human, animal and plant health, as long as these are based on science, are necessary for the

protection of health and do not unjustifiably discriminate among foreign sources of supply. Governments continue to determine the food safety levels and animal and plant health protection in their country.

The SPS Agreement does however encourage governments to "harmonize" or base their measures on international standards, guidelines and recommendation developed by WTO member governments in other international organizations. These organizations include, for food safety, the Codex Alimentarius Commission.

One problem with international standards is that they often set so stringent requirements that many countries find it difficult to implement them nationally. But the encouragement to use international standards does not mean that these will become a ceiling on national standards. National standards will not violate the SPS Agreement simply because they are higher than international norms. In fact, the SPS Agreement explicitly permits governments to impose more stringent requirements than those based on international standards. However governments which do not base their national requirements on relevant international standards may, if this difference gives rise to a trade dispute, be required to justify their higher standards.

The SPS Agreement allows countries to give food safety, animal and plant health priority over trade, provided there is a demonstrable scientific basis for their safety and health requirement. Each country has the right to determine what level of food safety and animal and plant health it considers appropriate, based on an assessment of the risks involved. Once a country has decided on its acceptable level of risk, there are often a number of alternative measures which may be used to achieve this protection.

Under the GATT agreement, Codex Standards, Guidelines and Recommendations have received increased importance which has given new direction to the work of the Codex Commission and its subsidiary bodies, including the Codex Committee on Milk and Milk Products.

The Codex Alimentarius Commission is made up of several subsidiary bodies or committees that develop standards, guidelines, and recommendations. These subsidiary bodies are included in four main areas:

1. Commodity Committees
2. General Subject Committees
3. Regional and Co-ordination Committees
4. Groups of Experts

The Codex Committee on Milk and Milk Products is a Commodity Committee and existed before there was a Codex Alimentarius Commission. It is the forerunner of all the food standards activities that have developed in recent years through Codex. This committee was originally known as the Joint FAO/WHO Committee of Government Experts on the Code of Principles concerning Milk and Milk Products and was established by FAO and WHO in 1958. It became a subsidiary commodity committee entitled the Codex Committee on Milk and Milk Products (CCMMP) in 1992.

The CCMMP has developed a Code of Principles for Milk and Milk Products, 13 general standards for dairy products such as butter, cheese, and milk powder and 35 standards for individual cheese varieties.

Each new or revised Codex standard follows a prescribed eight step procedure. This procedure allows countries to review and comment on standards developed by Codex.

- Step 1. The Commission or its Executive Committee decides that a standard should be elaborated and also which subsidiary body should undertake the work.
- Step 2. The Secretariat arranges for the preparation of a "proposed draft standard".
- Step 3. The proposed draft standard is sent to Members of the Commission and interested international organizations for comments.

- Step 4. The Secretariat forwards the comments received to the subsidiary body concerned to consider and to amend the proposed draft standard.
- Step 5. The Secretariat after first review may present text to the Commission as a "draft standard".
- Step 6. If the Commission adopts the draft standard it is sent by the Secretariat to all Members and interested international organizations for further comments, including implications on their economic interest.
- Step 7. The Secretariat forwards the comments received to the subsidiary body concerned to consider and to amend the draft.
- Step 8. The draft standard is submitted through the Secretariat to the Commission to adopt it as a "Codex Standard".

Once CCMMP became a subsidiary commodity committee of Codex, we were directed to review each milk and milk product standard and revise them to follow established Codex format. As you can imagine, this is a very formidable task. The CCMMP is currently reviewing each standard and revising them to comply with the following format:

1. Scope
2. Description
3. Essential Composition and Quality Factors
 - Raw Materials
 - Permitted Ingredients
 - Composition
 - Heat Treatment
4. Food Additives
 - Only those food additives listed in the Annex (or, when adopted, the Codex Standard for Food Additives) may be used within the limits specified.
5. Contaminants
 - Heavy Metals: The products covered by this standard shall comply with the maximum limits established by the Codex Committee on Food Additives and Contaminants.
 - Pesticide Residues: The products covered by this Standard shall comply with the maximum residue limits established by the Codex Committee on Pesticide Residues.
6. Hygiene
 - To the extent possible, in good manufacturing practice, the product shall be free from objectionable matter.
 - When tested by appropriate methods of sampling and examination, the product:
 - (a) shall be free from microorganisms in amounts which may represent a hazard to health;
 - (b) shall be free from parasites which may represent a hazard to health; and
 - (c) shall not contain any substance originating from microorganisms in amounts which may represent a hazard to health.
7. Labeling
 - Name of the Food
 - The name of the food shall be...
 - Labeling of Non-Retail Containers
 - Milk of Species other than the Cow
8. Methods of Sampling and Analysis

Many of the requirements contained in Codex Standards are similar to requirements effecting products in the United States. Some of these requirements are included in our Standards of Identity, while other are contained in other regulations effecting labeling, additive, and contaminant requirements. Just as these regulations dictate requirements for products marketed in the United States, Codex Standards provide requirements for products marketed internationally.

The CCMMP has held two sessions. The most recent session took place last May at FAO headquarters in Rome, Italy, and was hosted by the Government of New Zealand. Attendance totaled 203 delegates and observers, representing 55 countries and 5 international organizations. The 13 member delegation from the U.S. was made up of four government officials representing USDA and FDA. In addition, the Delegation included representatives from the International Dairy Foods Association, the American Dairy Products Institute, the National Milk Producers Federation, the U.S. Dairy Export Council, and three individuals representing various dairy companies. The agenda for this meeting included the following topics:

- Review of the Code of Principles Concerning Milk and Milk Products
- Consideration of Standards at Step 7
 - Butter
 - Milkfat Products
 - Evaporated Milks
 - Sweetened Condensed Milks
 - Milk and Cream Powders
 - Cheese
 - Whey Cheese
 - Cheeses in Brine
 - Unripened Cheeses
- Consideration of Standards at Step 3
 - Processed Cheese
 - Cream
 - Fermented Milk Products
 - Individual Cheese Standards
- Consideration of Heat Treatment Definitions
- Consideration of Nutritional and Quality Descriptors for Milk Products
- Consideration of the Draft Code of Hygienic Practice for Uncured/Unripened Cheese and Ripened Soft Cheese

We were able to complete our work and reach agreement on the General Standards for Cheese, Whey Cheese, and Cheese in Brine. These standards were advance to Step 8 of the Codex process.

We were not able to reach agreement on the General Standard for Unripened Cheeses and this document was retained at Step 6 for redraft and further discussion at the next session of the CCMMP. The reason this standard was not advanced was due to a request of the Italian Delegation to include Mozzarella cheese within its scope. Prior to this, the scope specifically excluded Mozzarella cheese from the standard. Because of this action, the U.S. supported retention of this standards at Step 6.

Because of the extensive agenda, the process cheese and individual cheese standards at Step 3 were not discussed. These standards will be redrafted in light of consequential decisions made with other standards and will be on the agenda for CCMMP's third session in 1998.

There are many issues to be debated at future meeting of the CCMMP. Some of the more critical cheese issues affecting the U.S. include:

- The development of Codex standards for Mozzarella cheese.

New work was accepted at the second session of CCMMP to develop standards for Mozzarella cheese. Currently, the International Dairy Federation, the technical advisor to the CCMMP, is developing an initial draft and we expect discussion of this draft to take place at the 1998 session.

- Restrictions placed on fat modified cheeses.

The Draft Code of Principles Concerning Milk and Milk Products contain information providing for a milk product that is modified in composition beyond the limits specified in a Codex standard. An exclusion is made for cheese and would not permit the international marketing of fat modified cheeses. At the second session of CCMMP, the U.S. expressed its concern with this exclusion and that cheeses be included. This issue is expected to be discussed again at the 1998 meeting.

- Revision of the Hygiene section of all milk and milk product standards to include pasteurization or equivalent.

At the last session, the U.S. suggested that the following wording be included in the Food Hygiene section of all milk and milk product standards: "Pasteurization or an equivalent measure approved by the official agency having jurisdiction, shall be used in order to achieve the appropriate level of public health protection."

Significant opposition to mandatory pasteurization was voiced by several of the European Delegations. CCMMP agreed to refer the proposed modification to the Codex Committee on Food Hygiene for consideration at future meeting of this committee.

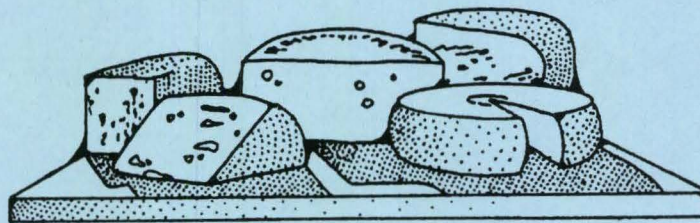
- Revision of Process Cheese standards.

The U.S. is the worlds largest producer of process cheese. The draft standard that was scheduled for discussion at the last session did not adequately reflect products produced in the U.S. A revision of this standard is expected to be on the agenda for the next session. The U.S. will be prepared to suggest revisions that will make this standard more consistent with our production practices.

Dairy manufacturers have a great deal at stake in the international market. Not only must they be concerned with establishing export markets, they must be also be aware of the impact of imports in our market. International standards establish the basis for this trade to take place. Industry involvement in the process of establishing international standards is encouraged not only by Codex, but also by the United States National Committee of the International Dairy Federation. Active participation by industry experts in international standards writing organizations will provide the much needed direction that will benefit the U.S. in international markets.

Duane R. Spomer, U.S. Delegate
Codex Committee on Milk and Milk Products
Dairy Division
Agricultural Marketing Service
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COMPONENT PRICING IN THE CHEESE INDUSTRY



CALVIN COVINGTON
AMERICAN JERSEY CATTLE ASSOCIATION

COMPONENT PRICING IN THE CHEESE INDUSTRY

Calvin Covington¹

The major production cost in manufacturing cheese is the cost of milk. For a plant manufacturing barrel or block cheese, milk represents between 85 - 90% of the total production cost. With milk being the predominant cost in making cheese, cheese makers have, or should have, a strong interest in milk pricing and in the method of how plants pay and producers are paid for milk.

One of the major developments in milk pricing during the past 20 years has been the change in the method of pricing milk. The industry has moved from a system of pricing milk based on skim or volume and butterfat to one that prices milk's skim portion based on components. This type of milk pricing is called component or more appropriately multiple component pricing (MCP). MCP is the pricing of milk based on two or more of milk's components. The components used are generally butterfat and either solids-not-fat (SNF), protein, and/or other solids.

In 1976, twenty years ago, only about 10% of the nation's milk supply was priced using some form of MCP. Almost all MCP pricing at that time was in the state of California. Most of the plans in use at that time priced the components butterfat and SNF. Today, twenty years later, 80% of the entire U.S. milk supply is eligible to be priced under some type of MCP plan. Seventy-seven and one half percent (77.5%) of the plans use the component protein. Twenty years ago it was thought to be almost impossible to change a federal milk marketing order from pricing milk based on volume and butterfat to one based on components. Today, 13 of the 33 federal orders have implemented MCP. Appendix 1 shows the widespread use of MCP plans.

The purpose of this presentation is to supply you, the cheese maker, with information on MCP. This information will hopefully help increase your understanding of MCP, distinguish the differences in the various types of MCP plans, analyze the impact of the various plans on your milk cost, look at the industry reaction to MCP, and discuss future considerations for MCP.

Background

MCP is a relatively new method of pricing milk in the United States (U.S.) compared to the other major milk producing countries in the world. Pricing of milk on a multiple component basis has been common throughout Europe for a number years. New Zealand and most of Australia adopted MCP about ten years ago. Canada's two largest milk producing provinces use MCP.

¹Calvin Covington, Chief Executive Officer, American Jersey Cattle Association-National All-Jersey Inc., 6486 East Main Street, Reynoldsburg, Ohio, 43068-2362 USA, Telephone 614/861-3636, FAX 614/861/8040. For presentation at the 12th Biennial Cheese Industry Conference, Logan, Utah, August 20-22, 1996.

The first regulated MCP plan in the U.S. started in California in 1962. However, MCP only applied to Class I products. It was not until 1969 that California extended the use of MCP to all classes. The 1970's saw a number of cheese plants and cooperatives implement their own voluntary MCP programs. These programs varied from plant to plant and from cooperative to cooperative. Most were just premium programs. A protein or SNF premium is paid in addition to a price based on volume and butterfat. These types of plans were not considered "true" MCP because they were premium programs with no deductions for low component milk and only priced components to producers not processors. Most are classified as producer payment plans.

Some cooperatives and cheese plants did start to implement "true" MCP plans in the late 1970's. Most of these plans were end product or cheese yield pricing, the system developed here at Utah State University by Dr. C. A. Ernstrom. This type of milk pricing system pays for milk based on the pounds of product or cheese that is manufactured from a hundredweight (cwt.) of milk. The 1980's saw further adoption and expansion of MCP plans. Again, except for California, most were voluntary or classified as producer payment plans.

It was not until 1988 that a MCP plan was implemented in a federal milk marketing order. The first federal order MCP plan was implemented in the Great Basin in April, 1988. The Great Basin is the order that covers this area. Since 1988, MCP plans have been implemented in 12 more orders. Currently, 13 of the 33 federal orders use MCP. These 13 orders market approximately 55% of all milk. Overall, Federal orders regulate approximately 70% of the U.S. milk supply.

Table 1 shows the current state and federal regulated milk orders with MCP, the year implemented, and components priced.

Table 1.

State and Federal Regulated Milk Orders with MCP

Order	Year Implemented	Components Priced
California	1962(Class I)	Butterfat, SNF
California	1969(all classes)	Butterfat, SNF
Great Basin	April 1988	Butterfat, Protein
Middle-Atlantic	January 1992	Butterfat, SNF
Eastern Ohio-Western Pennsylvania	October 1993	Butterfat, Protein
Ohio Valley	October 1993	Butterfat, Protein
Indiana	October 1993	Butterfat, Protein
Pacific Northwest	May 1994	Butterfat, SNF
Southwestern Idaho-Eastern Oregon	May 1994	Butterfat, Protein
Southern Michigan	October 1995	Butterfat, Protein, Fluid residual
Chicago Regional	January 1996	Butterfat, Protein, Other solids
Upper Midwest	January 1996	Butterfat, Protein, Other solids
Iowa	January 1996	Butterfat, Protein, Other solids
Nebraska-Western Iowa	January 1996	Butterfat, Protein, Other solids
Eastern South Dakota	January 1996	Butterfat, Protein, Other solids

Why the growth

As stated earlier, during the past twenty years, the use of MCP has gone from less than 10% of the milk supply to over 80%. Why the growth and acceptance? There are four primary reasons.

First is the expansion of dairy manufacturing, especially cheese. The traditional milk pricing system, volume or skim-butterfat, was established about 50 years ago and was designed for the fluid industry when fluid milk was the major use of milk. Cheese milk was considered a surplus use of milk. Today that has changed. In 1994, 64.5% of the nation's milk supply was used in manufacturing products. This is up from 55.6% in 1970. In 1970, 16.8% of the nation's milk supply was used in cheese manufacture. In 1994, it had increased to 33.2%. Table 2 shows the utilization of U.S. milk production on a milkfat basis from 1970 to 1994.

Table 2.

Utilization of U.S. Supply of Milkfat, 1970-1994

Year	Fluid	Cheese	Butter	Other
	(Percent of Total Butterfat Supply)			
1970	44.4	16.8	20.4	18.4
1980	39.6	26.4	17.7	16.3
1990	37.4	32.0	16.9	13.8
1994	35.5	33.2	16.0	15.3

Source: NMPF 1995 Dairy Producer Highlights
IDFA 1995 Milk Facts

A National Dairy Board report projects that per capita cheese consumption will increase from 27.3 lbs. in 1995 to almost 30 lbs. in 1999. On a whole milk equivalent basis, the Dairy Board projects 45% of the nation's milk supply will be utilized in cheese by 1999.

This shift in utilization combined with the fact that the value of the components in skim can be more easily quantified in manufactured products versus fluid dairy products, is a major reason for the greater use of MCP plans.

The second reason for the growth of MCP plans is the shift in milk's value from butterfat to skim. Since 1960, the relative values of skim and butterfat in the Minnesota-Wisconsin price series (M-W) now the Basic Formula Price (BFP) have reversed. The M-W, now the BFP, is the basic milk price for all federal orders. Table 3 shows this shift.

Table 3.

Skim and Butterfat Values of the M-W and BFP 1960-1995

Year	Average Yearly M-W or BFP (\$/cwt.)	Butterfat Differential	Skim Value %	Butterfat Value %
1960	\$3.13	\$0.068	23	77
1970	\$4.66	\$0.080	38	62
1980	\$11.88	\$0.160	51	49
1990	\$12.21	\$0.117	64	36
1995	\$11.83	\$0.070	77	23

Source: Dairy Market News and Federal Order Statistics

Table 3 shows a clearly dramatic shift has occurred in milk's basic value. The higher value on skim, coupled with increased manufacturing, has encouraged MCP expansion. Now that skim accounts for over three-fourths of milk's value, milk buyers have seen the need to pay for components in the skim portion of milk. In addition, MCP gives dairy farmers the economic incentive to increase the component levels within the skim portion rather than just volume.

The third reason is equity to both processors and producers. Skim-butterfat pricing places the same value upon milk that contains four pounds of protein as one that contains two pounds. When one considers the economic and functional value of the components contained in the skim portion, it is clear that skim-butterfat pricing is not equitable to either the milk producer or the milk buyer.

The need to give dairy farmers the proper economic incentive is the fourth reason for the expansion of MCP. Skim-butterfat pricing actually encourages production of volume or the term we like to use, cow water. This is shown in Table 4.

Table 4.

How Skim-Butterfat Pricing Encourages the Production of Cow Water

	Milk "A"	Milk "B"
Pounds milk	100.00	114.29
Butterfat %	4.00%	3.50%
Pounds butterfat	4.00	4.00
Protein %	3.50%	3.06%
Pounds protein	3.50	3.50
Pounds cheese	10.95	10.95
3.5% milk price	\$11.83	\$11.83
Butterfat differential	\$ 0.07	\$ 0.07
Total milk price	\$12.18	\$13.52
Milk cost per lb. cheese	\$1.11	\$1.23

The expansion of dairy manufacturing, the shift in milk's value from butterfat to the skim portion, treating both milk buyers and handlers equitably, and the need to give dairy producers the proper economic incentive are the major reasons for the growth and acceptance of MCP.

Variety of plans

Rather than one type of MCP plan, there are many plans in use today. Let us interject here that there is the perception that the only type of MCP plan is one that pays for milk based on pounds of components. This is incorrect. Any pricing plan that pays for two or more components is classified as a MCP plan. Table 5 shows there are a variety of such plans.

Table 5.

Types of MCP Plans

1. Variations of skim-butterfat pricing - skim is paid on a cwt. basis for milk containing 3.5% butterfat.

skim + butterfat differential + protein premium
skim + butterfat differential + SNF premium

2. Components paid on a per pound basis.

butterfat + protein
butterfat + SNF
butterfat + protein + other solids
butterfat + protein + fluid residual

Note: Some of these plans also pay the fluid differentials on a cwt. basis.

3. End Product or Cheese Yield Pricing

Predicted cheese yield x cheese yield value per lb.
(cheese price - make allowance) = milk price per cwt.

As seen in Table 5, there are a variety of MCP plans in use. Let us point out there are also variations of these plans. Many will have quality adjustments or requirements and other types of premiums.

Impact on the cheese industry

From the perspective of a cheese maker the most important question about MCP plans is how do they impact the cost of milk. Table 6 compares the milk cost per pound of cheese under various milk pricing plans. Figure 1 shows the results graphically. As you study this material, we want to emphasize the methodology of the plans and the variation on milk cost per pound of cheese rather than the level of prices. The price levels used in Table 6 are 1995 averages or estimates. These prices are shown in Table 7.

Table 6.

1995 Milk Cost per Pound Cheddar Cheese under Various MCP Plans

Milk Composition											
Fat %	3.00	3.20	3.40	3.60	3.80	4.00	4.20	4.40	4.60	4.80	5.00
Protein %	2.90	3.00	3.10	3.20	3.30	3.40	3.50	3.60	3.70	3.80	3.90
Other solids %	5.40	5.41	5.42	5.43	5.44	5.45	5.46	5.47	5.48	5.49	5.50
Solids-not-fat %	8.30	8.41	8.52	8.63	8.74	8.85	8.96	9.07	9.18	9.29	9.40
Fat % in cheese*	3.00	3.20	3.40	3.60	3.80	4.00	4.20	4.39	4.51	4.63	4.75
Cheese yield**											
	8.55	9.00	9.45	9.91	10.36	10.82	11.27	11.71	12.03	12.36	12.69
Excess fat***											
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.09	0.17	0.25
Milk Pricing Plans****											
Skim + fat	\$11.48	\$11.62	\$11.76	\$11.90	\$12.04	\$12.18	\$12.32	\$12.46	\$12.60	\$12.74	\$12.88
Milk Cost per Lb. Cheese	\$1.34	\$1.29	\$1.24	\$1.20	\$1.16	\$1.13	\$1.09	\$1.06	\$1.04	\$1.02	\$1.00
Skim + fat + protein premium	\$11.48	\$11.62	\$11.76	\$11.90	\$12.19	\$12.48	\$12.77	\$13.06	\$13.35	\$13.64	\$13.93
Milk Cost per Lb. Cheese	\$1.34	\$1.29	\$1.24	\$1.20	\$1.18	\$1.15	\$1.13	\$1.11	\$1.10	\$1.09	\$1.08
Fat + Solids-not-fat	\$11.09	\$11.36	\$11.63	\$11.91	\$12.18	\$12.45	\$12.73	\$13.00	\$13.27	\$13.55	\$13.82
Milk Cost per Lb. Cheese	\$1.30	\$1.26	\$1.23	\$1.20	\$1.18	\$1.15	\$1.13	\$1.11	\$1.10	\$1.08	\$1.07
Fat + protein + fluid residual	\$10.95	\$11.27	\$11.59	\$11.91	\$12.23	\$12.55	\$12.86	\$13.18	\$13.50	\$13.82	\$14.14
Milk Cost per Lb. Cheese	\$1.28	\$1.25	\$1.23	\$1.20	\$1.18	\$1.16	\$1.14	\$1.13	\$1.12	\$1.11	\$1.10
Fat + Protein + Other Solids	\$10.92	\$11.26	\$11.60	\$11.93	\$12.27	\$12.61	\$12.94	\$13.28	\$13.61	\$13.95	\$14.29
Milk Cost per Lb. Cheese	\$1.28	\$1.25	\$1.23	\$1.20	\$1.18	\$1.17	\$1.15	\$1.13	\$1.13	\$1.12	\$1.11
Fat + Protein	\$10.58	\$11.02	\$11.46	\$11.90	\$12.34	\$12.78	\$13.22	\$13.66	\$14.11	\$14.55	\$14.99
Milk Cost per Lb. Cheese	\$1.24	\$1.22	\$1.21	\$1.20	\$1.19	\$1.18	\$1.17	\$1.17	\$1.17	\$1.17	\$1.17
End Product Pricing	\$10.27	\$10.81	\$11.36	\$11.90	\$12.45	\$12.99	\$13.54	\$14.07	\$14.53	\$14.99	\$15.44
Milk Cost per Lb. Cheese	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20	\$1.20

* The fat content in the raw milk supply is adjusted not to fall below a casein:fat ratio of 0.64:1.

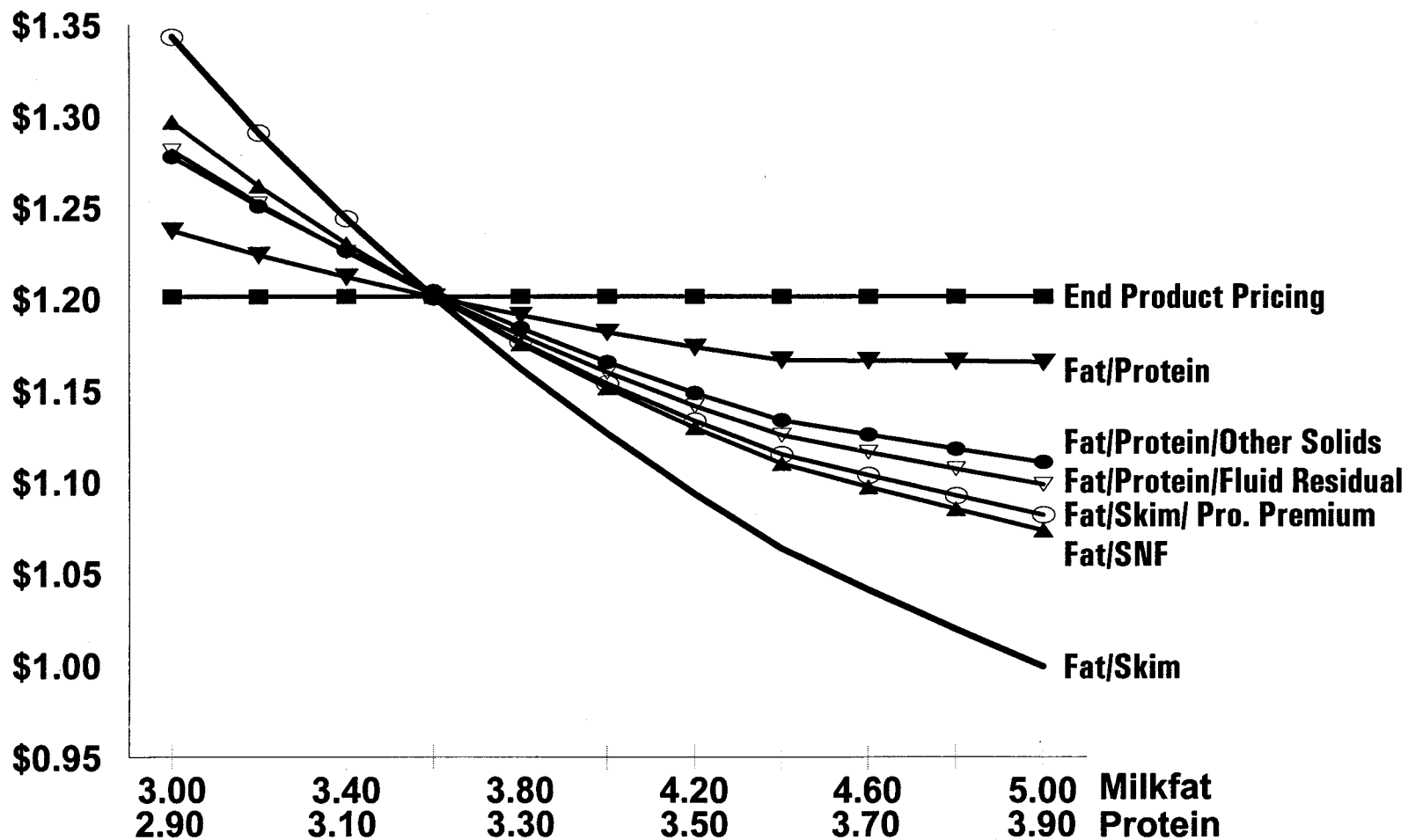
** A modified Van Slyke Formula for cheddar cheese is used for this cost comparison: $(.90\% \times \text{Fat Used in Cheese} + 78\% \times \text{Milk Protein} - 0.1) \times 1.09$
(1 - 38% Moisture)

*** Excess fat equals the amount of fat in raw milk, less the amount of fat used in cheese.

**** Milk pricing plans based on actual component price averages for 1995. Protein premium equals 15 cents per point protein over 3.2%. Cheese Yield is calculated using the average 1995 BFP implied make allowance of \$0.103 per pound cheese yield. Milk cost per pound cheese adjusted for excess milkfat at the Federal Order Value.

Figure 1.

1995 Milk Cost Per Pound Cheddar Yield For Seven Different Milk Pricing Plans



Source: USDA and National All-Jersey, Inc.

Under all plans, the price levels at average test are almost identical. This can be seen by looking at the prices paid for milk at average component levels, 3.6% fat, 3.2% protein, and 8.63% SNF. The point to remember is that, initially, any MCP plan does not change the total dollars available to pay for milk. MCP changes how the dollars are distributed. MCP, or for that fact, any pricing system does not determine the total dollars a cheese plant pays for milk. It determines how the dollars will be distributed. This is an extremely important concept to remember when studying MCP plans and one that many overlook.

Table 7.
Prices Used to Determine Milk Cost Per Pound of Cheese for Various MCP Plans
(Prices are 1995 Averages or Estimates)

Plan	Component	Price Per Pound or Per Cwt.
Skim+BF	Skim	\$11.83/cwt.
	BF	\$0.07/point
Skim+BF+Protein Premium	Skim	\$11.83/cwt.
	BF	\$0.07/point
	Protein	\$0.15/point
BF+SNF	BF	\$0.79/lb.
	SNF	\$1.05/lb.
BF+Protein+Fluid Residual	BF	\$0.79/lb.
	Protein	\$1.72/lb.
	Fluid Residual	\$0.0382/lb.
BF+Protein+Other solids	BF	\$0.79/lb.
	Protein	\$1.72/lb.
	Other solids	\$0.66/lb.
BF+Protein	BF	\$0.79/lb.
	Protein	\$2.83/lb.
End Product Pricing		\$1.20/lb. cheese

Table 8 is a condensed version of Table 6. In the condensed table one can more easily see the range in milk cost per lb. of cheese under the various types of MCP plans.

Table 8.

**Milk Cost Per Pound of Cheese Under Various
Milk Pricing Plans for Three Component Levels**

<u>Component Level</u>				<u>Range</u>
Protein %	2.90	3.20	3.90	1.00
Protein %	2.90	3.20	3.90	1.00
Other Solids %	5.40	5.43	5.50	0.10
SNF %	8.30	8.63	9.40	1.10
<u>Type of Pricing Plan</u>				<u>Range</u>
Skim+Butterfat (BF)	\$1.34	\$1.20	\$1.01	\$0.34
BF+SNF	\$1.30	\$1.20	\$1.07	\$0.23
BF+Protein+Fluid Residual	\$1.28	\$1.20	\$1.10	\$0.18
BF+Protein+Other solids	\$1.28	\$1.20	\$1.11	\$0.17
BF+Protein	\$1.24	\$1.20	\$1.17	\$0.07
End Product Pricing	\$1.20	\$1.20	\$1.20	\$0.00

Skim and butterfat pricing, which is not a MCP plan, is included to show that all MCP plans in use are an improvement on this traditional pricing plan. For example, milk cost per pound of cheese under skim-butterfat varies from \$1.34 per pound to a \$1.01 per pound. This is a range of \$.34 per pound of cheese.

All MCP plans shown lessen the range of milk cost per lb. of cheese compared to skim-butterfat pricing. However, some have a smaller range of milk cost per pound of cheese than others. For example, of the current MCP plans used in federal orders, butterfat and SNF has the greatest range which is from \$1.30 to \$1.07 per pound of cheese. Butterfat and protein has the lowest range, which is from \$1.24 to \$1.17 per pound. However, the only pricing plan that has the same milk cost per pound of cheese across milks of all component levels is end product pricing.

Tables 6 and 8 and Figure 1 show explicitly that the actual milk cost per pound of cheese is not the same under all milk pricing plans including MCP plans. As a cheese maker we assume that when you look at your actual milk cost, which is 85-90% of production cost, you look at it on a per pound of cheese basis.

Again, we want to point out that if different price levels were substituted into Tables 6, the ranges between the various pricing plans will remain the same. If the milk price was increased \$3.00 per cwt. or lowered \$3.00 per cwt. the relative difference on a per pound of cheese basis would remain the same. In addition, if the cheese yield for other types of cheeses, for example mozzarella, were used instead of cheddar the results would be similar.

My suggestion to you as cheese makers in deciding what type of milk pricing plan you want to implement is to think about what you want to accomplish with a milk pricing system other than just paying for milk. The following are points to remember and consider:

- ◆ For a plant taking in average milk, the total dollars paid for milk, initially, are almost the same under any milk pricing system.
- ◆ Does the milk pricing system allow the plant to have similar or different milk costs per pound of cheese across all component levels?
- ◆ What incentive is given to milk producers? Do milk producers have the incentive to increase or decrease the potential cheese yield of their milk? Cheese yield is a major factor in cheese plant efficiency.

These are areas we encourage you to consider regarding your milk pricing system.

Industry reaction

A common question we receive about MCP is how has the industry reacted to MCP. In general, both the processors and producers have responded positively.

From the processors viewpoint the best example is almost every plant or cooperative that has implemented some type of MCP plan is still using the plan. In most cases the plans have been refined and improved. We know of only a few MCP plans stopped once started.

A recent article that appeared in the July 5, 1996 issue of *Cheese Market News* discussed the industry reaction to the implementation of MCP on January 1, 1996 in five Upper Midwest federal orders. According to the article almost all industry responses were positive. The following are some of the reactions from cheese plant and cooperative management:

"...the transition to multiple component pricing has gone well."

"...biggest pay-off has been that it translates the market value of milk to producers."

"...doing exactly what it's supposed to do-it lets producers see the individual components in the milk they sell."

"...is a move in the right direction to provide market signals to producers."

"...reflects a market driven industry, rather than the government regulated industry of the past."

"...bringing real equity to the industry."

From the producers' side, more and more information is now available in the areas of genetics and management to assist dairy farmers in maximizing their income from MCP pricing. We are starting to see more top artificial insemination bulls that transmit higher component production. It appears dairy producers are responding to the signals sent by MCP pricing.

A headline in the June 17, 1996 *Dairy Profit Weekly* read "MCP boosts protein levels". The article stated, "The early read on multiple component pricing plans is that producers are responding by increasing protein levels ...". The market administrator for the Ohio Valley and Eastern Ohio-Western Pennsylvania federal orders stated in the article that protein levels in these two orders have been increasing 0.02 percent per year since the MCP plans were implemented in October 1993. It appears producers are responding to the signals sent by MCP.

Future considerations

What can be expected from MCP in the future? We are optimistic that MCP will be the method of pricing all milk in this country in the next few years. The increasing use of milk in manufacturing, especially cheese, the importance of the components contained in the skim portion of milk, and the need to have a milk pricing system that gives dairy producers the proper economic signal will move all milk pricing in this direction.

Further, Congress has put its stamp of approval on MCP. The new farm legislation, Federal Agricultural Improvement and Reform Act of 1996, which was enacted on April 4 of this year contains a provision on MCP. In fact, MCP is one of only three specific pricing provisions contained under the federal order reform section. This section mandates the consolidation of all federal orders into no less than ten or no more than fourteen orders. Based on MCP's inclusion in this legislation, proposals from various industry groups, and with MCP now being used in over half of all federal orders, it is a strong possibility that MCP will be included in all if not most proposals for order consolidation.

The major question now is not if MCP, but what type of plan? Almost every public hearing held to consider implementing MCP into a regulated pricing system has seen a refinement in the type of pricing system used. We have seen the shift from determining the protein value from one based on a residual of the BFP to one based on product yield. We expect this trend to continue.

Another consideration for the future is the world market. Internationalization is occurring in the dairy industry. We need to be cognizant of what is happening outside the U.S. in regards to milk pricing and act accordingly. As stated earlier all major milk producing countries in the world, except for the U.S., use MCP to price all producer milk. In fact, in New Zealand and parts of other countries, a negative value is placed on volume. The purpose of this negative value is to encourage producers to produce the maximum amount of milk solids in the least amount of volume. This helps the New Zealand dairy industry improve its efficiency and be more competitive.

As the debate and discussion on the type of MCP plan to use continues we offer three items that the industry should consider in deciding on the type of plan. Any MCP plan should be:

1. Equitable to all milk producers.
2. Equitable to all milk buyers.
3. Give the dairy producer the proper economic incentive to produce the kind of milk components processors and manufacturers need to economically produce the kind of dairy products consumers demand.

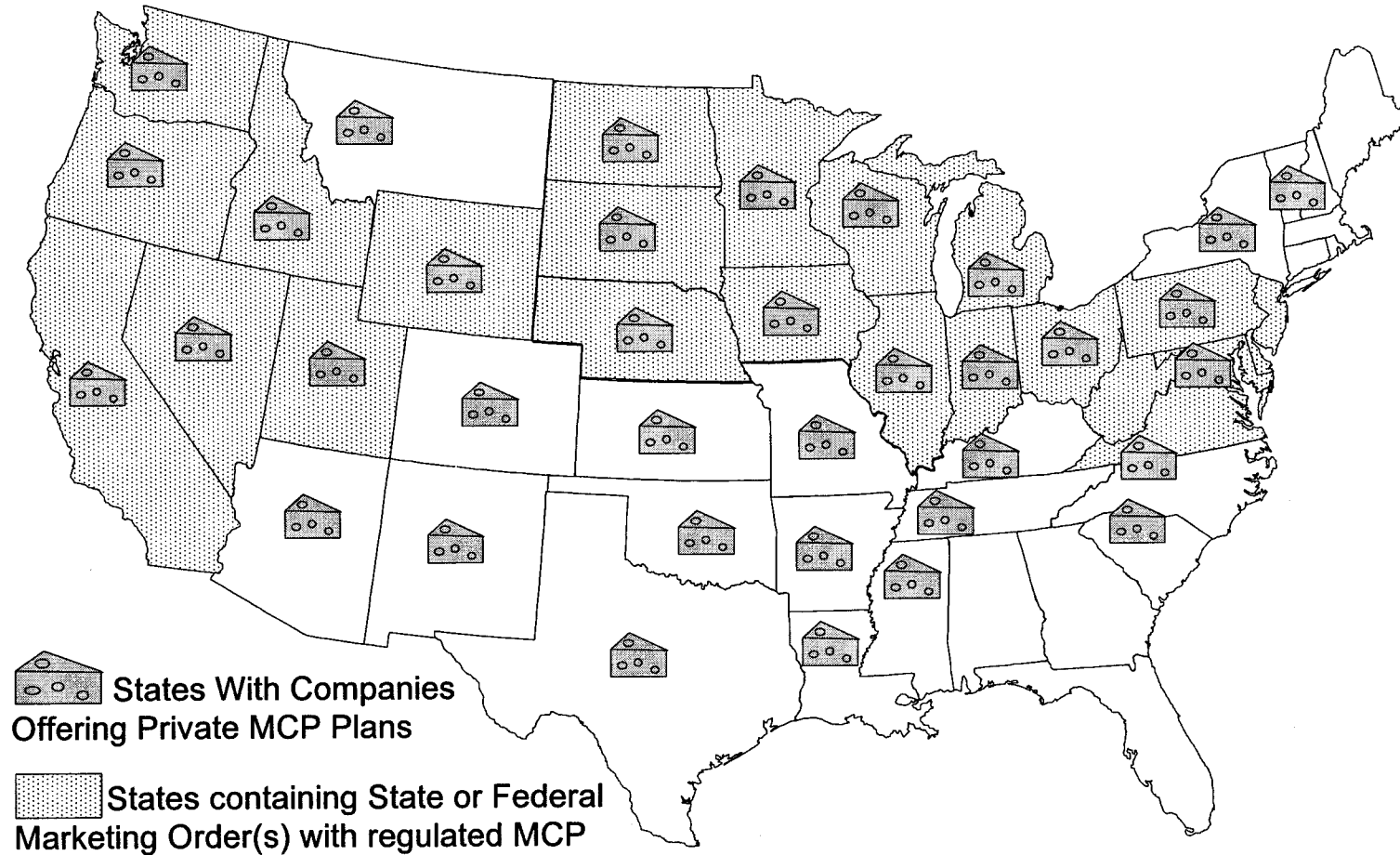
We consider the third reason to be the most important. All of us in the dairy industry must remember that it is the consumer of dairy products who keeps us in business. To have a strong dairy industry, dairy producers must produce the kind of milk components you as manufacturers need to efficiently provide consumers with the kind of dairy products they demand at the best value possible. In order for dairy farmers to do this, the method used to pay producers for their milk production must give producers the proper economic signal. This is the ultimate purpose of a pricing system - to pass along the proper incentives to the producers in order to accommodate the needs of the consumers.

Summary

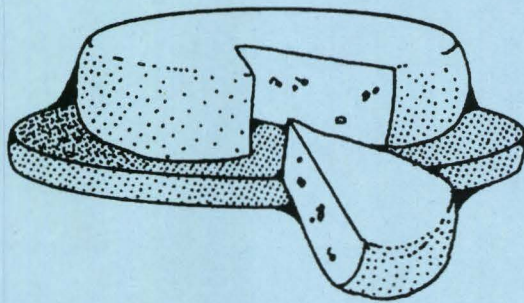
During the past twenty years we have seen the use of MCP grow from less than 10% of the nation's milk production being priced under some type of MCP to over 80% today. There are a variety of MCP plans in use. In analyzing the plans, cheese makers should focus on the milk cost per pound of cheese under the various types of plans. The most important factor to consider in deciding on the best MCP plan is make sure it gives dairy farmers the proper economic incentive. All signs point to MCP being used to price all of the nation's milk in the near future.

Appendix 1.

States Containing Regulated or Industry-Based Multiple Component Pricing (MCP) Plans

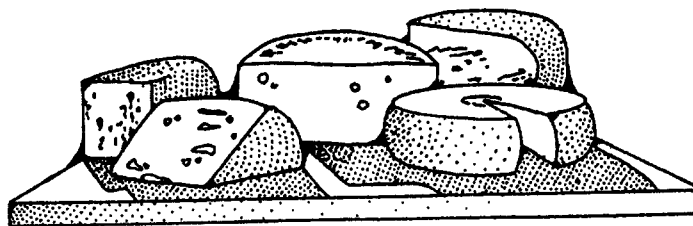


IMPORTANCE OF CURD AND CHEESE pH



**DAROLD JOHNSON
CHEESE TECHNOLOGY, INC.**

IMPORTANCE OF CURD AND CHEESE Ph



DAROLD JOHNSON
CHEESE TECHNOLOGY, INC

The Science and Practice of **Cheese-Making**

Lucius L. Van Slyke, Ph.D.

*Chemist of the New York Agricultural
Experiment Station*

and

Charles A. Publow, A.B., M.D., C.M.

*Associate Professor of Dairy Industry in the New York State
College of Agriculture at Cornell University*

New York

Orange Judd Company

1909

THE HOT-IRON TEST

This test is used for the purpose of ascertaining when to remove whey from curd and when to mill curd. An iron of convenient size and length for holding, as a half-inch gas-pipe, is heated fairly hot at one end. The iron is carefully wiped with a cloth until it is clean and smooth. A handful of curd is then taken and placed in dry cloth and squeezed by the

hand, until the surface has been well dried. The curd is then gently pressed against the portion of the iron where it is hot enough to make the curd stick to the iron but not hot enough to scorch it. The curd is then carefully drawn away from the iron and, if in proper condition, produces fine, silky threads, the length of which depends upon the amount of acidity of the curd.

Use of Quinhydrone Electrode for Following Changes of pH in Swiss Cheese¹

By Paul D. Watson

BUREAU OF DAIRY INDUSTRY, WASHINGTON, D. C.

1927

IT HAS long been known that the rapid development of acidity in a cheese, due to the formation of lactic acid from the lactose while the cheese is on the press, is an important factor in its subsequent ripening. Yet no information clearly showing this change is available in the literature, except the results of titrations of water extracts of cheese, and curves showing somewhat similar changes in the souring of milk.

Recently Knudsen² has published a method for measuring the pH of cheese by means of the quinhydrone electrode and has tabulated measurements on several varieties of mature cheese. Barthel and Haglund,³ using this method, have studied the pH changes on two cheeses for several days, but made only a few measurements. In 1926 the writer independently began to study the pH changes in green Swiss cheese by utilizing the Clark type of shaking hydrogen electrode for measurements upon whey which was extracted at intervals from the cheese. This method gave good results, except that the measurements had to be terminated in about 3 hours because of the difficulty of obtaining sufficient whey for the electrode vessels.

The quinhydrone electrode was then employed, by means of which changes of pH in cheese may be followed closely, and very interesting data were obtained quickly and accurately. It is the purpose of this paper to present briefly the technic employed, together with several graphic illustrations of the results it is possible to obtain with Swiss cheese. The theory of the quinhydrone electrode and details regarding the necessary materials and equipment may be found in many journals, some of which are listed in the references.

Apparatus

The capillary quinhydrone electrode of Cullen and Billmann⁴ and the cheese quinhydrone electrode of Knudsen have been combined in these measurements, the former being utilized for the 3- or 4-hour period after dipping, when it was possible to extract whey from cheeses weighing about 60 pounds, and the latter on samples of the cheese itself during the remainder of the run. The two types of electrodes are illustrated in Figure 1.

The electrodes first used were blades and wires of platinum and were cleaned between determinations by immersing them in sulfuric-chromic acid solution, washing in distilled water, and flaming in an alcohol lamp as recommended by Billmann.⁵ After a few weeks the electrodes began to give very erratic results, showing a rapid drift and giving a pH reading of 3.86 instead of 3.97 with 0.05 *M* acid potassium

phthalate. The electrodes were so badly poisoned that it was impossible to obtain correct values by any treatment until they were gold-plated, after which they performed satisfactorily. Therefore every few days, or when evidence of abnormality occurred, the old gold deposit was removed by electrolyzing a few seconds in dilute hydrochloric acid solution, and a new surface was secured by replating in a potassium auricyanide solution for a few seconds, using a current of 6 volts. When freshly plated the electrodes gave a pH of 3.97 with 0.05 *M* acid potassium phthalate solution, but occasionally they gave a pH of about 4.0. If allowed to stand in nitric acid, or after using once, it

would then give the correct value. After daily use for a week or more the readings with the phthalate would drop to around a pH of 3.92. The electrodes were cleaned between determinations by immersion in concentrated nitric acid and were then rinsed in distilled water. The blades were scrubbed with soap and water and a small brush before they were placed in the acid.

They were allowed to remain in the acid during each night when in daily use. The capillary glass tubes were cleaned and dried after each determination.

Pure gold electrodes were also used and found to be very satisfactory. They were occasionally replated with a new surface of gold and cleaned in a manner similar to the gold-plated platinum electrodes, but it was not necessary to replate so often. In order to secure desirable rigidity in the electrodes it is advisable to have them made from wire no smaller than B. & S. gage No. 19 for the platinum and No. 21 for the gold. The blades may be made of foil about 0.01 inch (0.25 mm.) thick.

The gold electrodes may be made by fusing the gold wire to platinum wire, which is then sealed into the glass tube, as it is necessary to have the glass sealed tightly around the wire. It is convenient to have about five wire electrodes and five blades in order that several measurements may be made on each sample and so that a new electrode may be quickly substituted for one giving an abnormal potential.

Before the electrodes were dipped into the potassium chloride bridge, they were connected to the potentiometer wires to which Fahnestock battery clips had been soldered. The connections between the electrodes and the potentiometer were made by means of a multiple switch.

The potentials were measured by means of a saturated calomel electrode, Leeds & Northrup Type K potentiometer, and portable galvanometer. The hydrogen electrode measurements on the whey were made with the Clark shaking electrode.

Very little attention has been directed towards the changes in hydrogen-ion concentration occurring in cheese. A technic is described in this paper for following these changes quickly and accurately. In this technic two types of the quinhydrone electrode are utilized for measurements upon the extracted whey and plug samples. It is shown that the two methods give comparable results, and their value in the control of the cheese-making process is indicated.

¹ Received July 9, 1927.

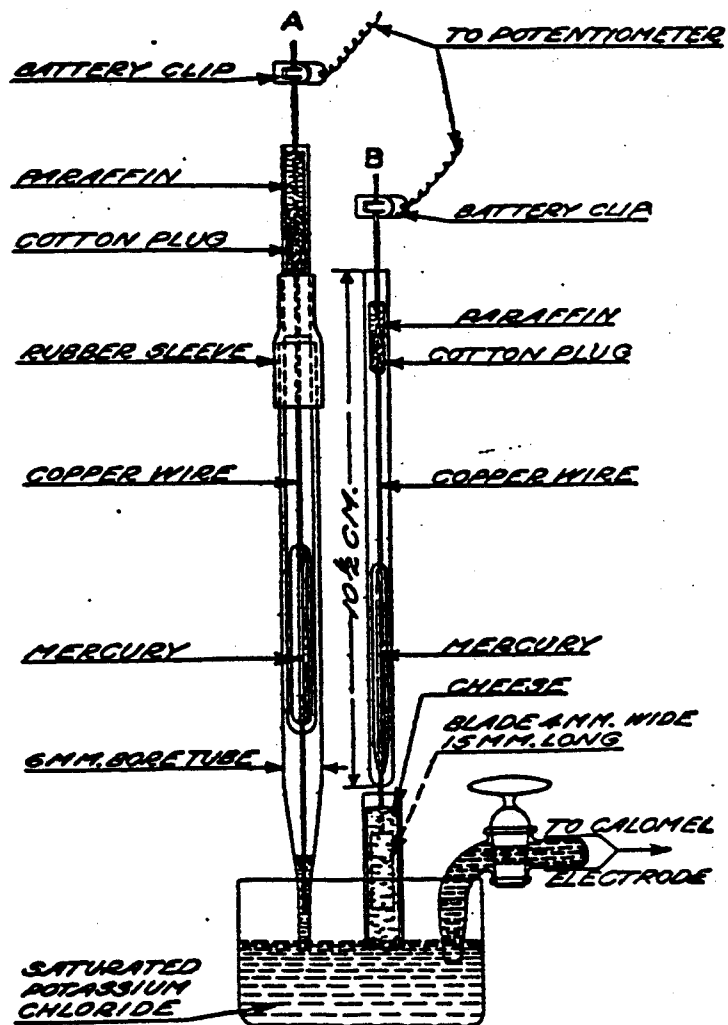
² *Z. Untersuch. Lebensm.*, **60**, 300 (1925).

³ *Medd. Centralanstalt. för sööksäsendet jordbruks.*, No. 307 (1926).

⁴ *J. Biol. Chem.*, **64**, 727 (1925).

⁵ *J. Agr. Sci.*, **14**, 232 (1924).

PAUL D. WATSON



TYPES OF THE QUINHYDRONE ELECTRODE USED FOR THE MEASUREMENT OF
HYDROGEN-ION CONCENTRATION
, Cullen and Büllmann capillary electrode; B, Knudsen cheese electrode

THE RELATION OF THE HYDROGEN-ION CONCENTRATION TO THE TEXTURE OF EMMENTHAL OR SWISS CHEESE*

PAUL D. WATSON

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INTRODUCTION

The object of the work described herein was the determination of the relation of the march of hydrogen-ion concentration in Emmenthal or Swiss cheese to the texture. In this research differences in the proteolysis and the moisture of the cheese were correlated with the above factors. For the purpose of comparative study variations were produced by the use of two starters, the *ga* starter, a *bulgaricus* organism with a mycoderm, and the *59a* starter, an old strain of *Lactobacillus bulgaricus*.

Orla-Jensen (1) has studied the influence of the degree of acidity upon the consistency and ripening process of Emmenthal cheese and this work will be referred to later. Boekhout and Ott de Vries (2) found that an excessive amount of free lactic acid was the direct cause of brittle texture in the Edam cheese. Van Dam's (3) researches on Edam cheese indicated that the texture was a phenomenon of colloidal chemistry, and that an excess of hydrogen ions also produced a hard and brittle texture. He also found that the progress of ripening was dependent upon the proportion of acid present. Leitch (4) who worked with English Cheddar cheese stated that the degree of acidification of the curd was the factor which exercised the greatest control over the texture of the cured cheese. In an investigation of Cheddar cheese Van Slyke and Hart (5) found that excess acid produced a dry, harsh, and hard texture and that cheese having the most moisture generally contained the largest amounts of soluble nitrogen. Van Slyke

* Received for publication December 7, 1929. Presented before the Division of Agricultural and Food Chemistry at the Seventy-sixth Meeting of the American Chemical Society, Swampscott, Mass., September 10 to 14, 1928.

CHEDDAR CHEESE

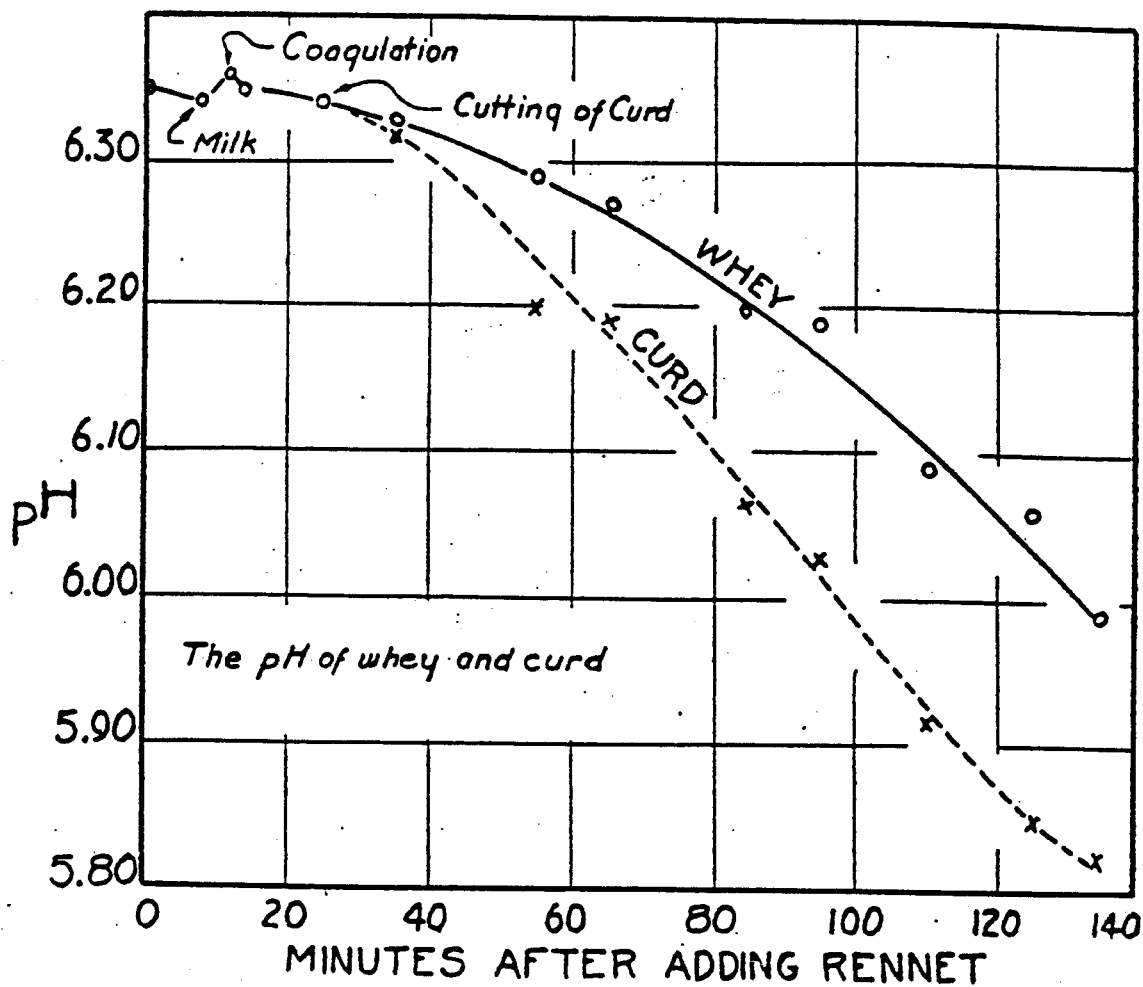


Figure 3

A STUDY OF THE RELATIONSHIPS BETWEEN HYDROGEN ION CONCENTRATION, TITRATABLE ACIDITY, AND QUALITY IN CHEDDAR CHEESE

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H.H. SOMMER-REVISED BY W.C. WINDER

Methods of Expressing Hydrogen Ion Concentration:-

We have already emphasized that in all mass law calculations, concentrations are expressed in terms of moles per liter. The conventional symbols to express all this are the particular molecule or ion enclosed in brackets; thus, (CH_3COOH) , (OH^-) , (H^+) , (Ca^{++}) , etc.

Thus, $(\text{H}^+) = 6.5 \times 10^{-6}$ says as follows:- The hydrogen ion concentration in moles per liter is 6.5×10^{-6} . This numerical value might also be written:-

$$\frac{6.5}{1,000,000}$$

Numerical values of this general order can be expressed more briefly, and for many purposes to better advantage, in terms of logarithms.

$$\text{Log}_{10} 6.5 \times 10^{-6} = \text{Log}_{10} \frac{6.5}{1,000,000} = \text{Log}_{10} 6.5 - \text{Log}_{10} 1,000,000$$

$$\text{Log}_{10} 6.5 = 0.81291$$

$$\text{Log}_{10} 1,000,000 = 6.00000$$

$$\therefore \text{Log}_{10} 6.5 \times 10^{-6} = 0.81291 - 6.00000 = -5.18709$$

$$\text{and } -\text{Log}_{10} 6.5 \times 10^{-6} = 5.18709 \quad (\text{To make the number positive})$$

By definition we call the $-\text{Log}_{10} (\text{H}^+)$, pH (P_H also used). In other words, pH is the negative logarithm of the hydrogen ion concentration.

To convert pH values into (H^+) proceed according to the following illustration:-

Convert pH 6.8 to (H^+) .

From the definition of pH we know that $\text{pH} = -\text{Log}_{10} (\text{H}^+)$. Therefore, $\text{Log}_{10} (\text{H}^+) = -6.8$ in this problem. We must now find the antilog of -6.8 , but since we cannot use the logarithm tables for negative logarithms, we must first convert this to a workable form. In this the fractional part of a unit must be positive. Thus, $0.2 - 7.0 = -6.8$. The antilog of 0.2 is 1.585. The antilog of -7.0 is $1/1,000,000$ or 10^{-7} . The antilog of -6.8 is 1.585×10^{-7} . Hence, $\text{pH } 6.8 = (\text{H}^+)$ of 1.585×10^{-7} .

The student who is familiar with logarithms should have no difficulty in transforming pH into (H^+) , and vice versa.

range	pH	H ⁺ concentration (mol/l)	OH ⁻ concentration (mol/l)
acid	0	1	0,0000000000000001
	1	0,1	0,00000000000001
	2	0,01	0,0000000000001
	3	0,001	0,000000000001
	4	0,0001	0,00000000001
	5	0,00001	0,0000000001
	6	0,000001	0,00000001
neutral	7	0,0000001	0,0000001
alkaline	8	0,00000001	0,000001
	9	0,000000001	0,00001
	10	0,0000000001	0,0001
	11	0,00000000001	0,001
	12	0,000000000001	0,01
	13	0,0000000000001	0,1
	14	0,00000000000001	1

SECOND
EDITION
R. SCOTT

pH Values

Over many years cheesemakers have become used to titratable acidity readings which can be carried out in or near the processing area and, because of constant usage, interpretation of the readings have been understood. pH measurements have been accepted slowly by cheesemakers partly because earlier pH instruments tended to be somewhat erratic, but newer instruments are more stable. Some source of error may arise from poisoning of the electrode by protein, fat or salts due to their adsorption on the glass surfaces, which is sometimes difficult to remove.

The glass electrode itself may suffer from scratching or etching of the glass, and care is necessary in both use and storage of the delicate electrodes.

Errors due to the electrodes occur with both acid and alkaline electrodes. Since the glass composition of the membrane influences the characteristics of the electrode, it is necessary to choose the appropriate electrode for the measurement in hand.

The pH value is a measure of the hydrogen ions dissociated in a solution. It does not measure the acidity as, for instance, by a titration.

Since the pH of a solution varies with temperature it is necessary to standardize the electrodes within a narrow pH range and at a fixed temperature.

The electronic method of measuring pH is dependent on measuring a very small potential (voltage) difference, between the glass electrode and a reference electrode that maintains a standard potential.

The reference electrode is composed of a potassium chloride solution (usually saturated) containing a calomel-mercury electrode. A saturated solution is stable to temperature changes, but other strengths of solution

are used. Other solutions, e.g. silver chloride, may also be used. This reference electrode must be maintained in good condition, since a dry area in the calomel-mercury disrupts measurement of potentials. The glass electrode also introduces errors if not properly 'wetted' and cleaned.

Soaking for a short time in $\frac{N}{10}$ hydrochloric acid followed by adequate rinsing in water avoids most difficulties due to dry or coated electrodes.

Alcoholic solutions sometimes used to remove fat can cause serious errors by 'drying out' the glass membrane.

The following are some difficulties encountered in the dairy industry when using electronic pH equipment.

- (1) Error due to moisture in the meter or wire leading to the meter. (Steamy atmosphere in the cheese room.)
- (2) Error due to static electricity or to lack of grounding (earthing) of the equipment when near to heavy duty electrical networks.
- (3) Sample errors. Colloids sensitive to salt concentrations (milk) may be precipitated at the liquid junction by leakage of electrolyte. The colloids may form a film on the glass electrode bulb. (Milk stone scale.)
- (4) Errors due to remains of a previous sample on the electrode, i.e. fat, proteins, etc.
- (5) Cracks in the glass of the electrode and leakage of the internal filling solution.
- (6) Electrode standardized at the wrong temperature range.
- (7) Transferring too quickly from one solution to another without allowing equilibrium to be established. (This is a typical error when transferring from vat sample to vat sample.)
- (8) Electrode glass composition error due to using the wrong type of electrode for the pH range.

Amplification of minute potential differences has now been developed to a high degree, but the more sensitive meters may cause difficulty in stabilization in a medium which itself is changing (i.e. milk curd, etc.).

The electrode systems are also liable to drift in their characteristics over a period of time and, therefore, frequent standardization is required to correct this drift as well as for temperature differences.

When measuring the pH values of moist curds the measurements may be more in respect of the whey exuded from the curd itself.

A moist 'spear' type electrode is more useful for measuring the pH of solid curds, since emulsification of curd in water, in order to dip the

globular glass electrode in the emulsion, is likely to introduce errors or instability.

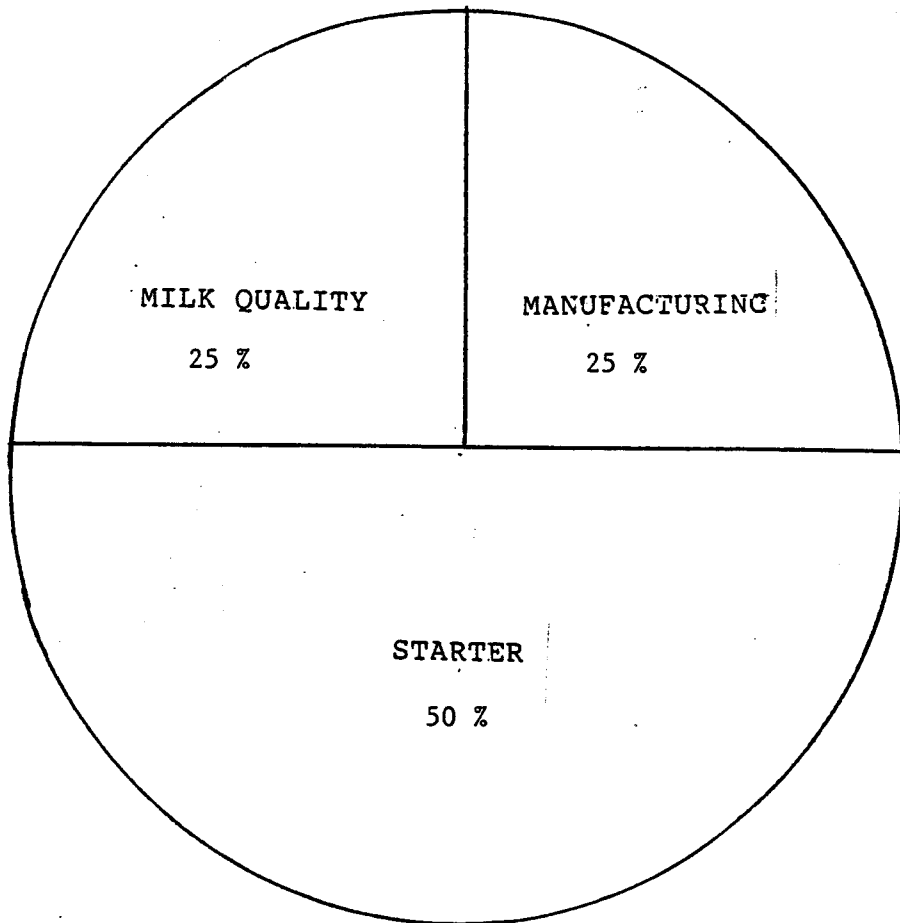
After heat treatment of milk and also after injection of calcium chloride solutions into the milk, changes in the equilibrium of the milk occur such that serious errors may be introduced, and time must elapse before satisfactory readings are taken.

The electronic pH meter readings, although almost instantaneous, may still need interpretation due to the high-buffer capacity of milk and curd.

In spite of some of these problems, pH values can be measured throughout the cheesemaking process from milk to final cheese and this is a major advantage of the system.

The pH value is a measure of the ionic dissociation in a solution and therefore measures the reactivity of the elements in that solution. It is, therefore, a value which affects biological or biochemical reactions, as opposed to the titratable acidity in the solution which involves buffer capacity of milk (up to pH 8.3). There is no correlation between pH values and acidity measurements, so that a cheesemaker cannot switch from one form of measurement to another.

SECOND
EDITION
R. SCOTT



**Emmentaler Cheese
Swiss Cheese Seminar
April 24, 1996**

**Wisconsin Center for Dairy Research
April 18, 1996**

Cheese Starter Cultures

T. M. Cogan

*The Agricultural Institute, Moorepark Research Centre,
Fermoy, Co. Cork, Ireland*

and

C. Daly

*Department of Food Microbiology, University College,
Cork, Ireland*

Acid production affects several aspects of cheese manufacture, besides gel syneresis, e.g.

1. Coagulant activity during coagulation.
2. Denaturation and retention of the coagulant in the curd during manufacture; this affects subsequent proteolysis during ripening and consequently, cheese flavour and texture.
3. Curd strength, which influences cheese yield.
4. The extent of dissolution of colloidal calcium phosphate, which modifies the susceptibility of casein to proteolysis and influences the rheological properties of the cheese, e.g. compare the texture of Emmental, Gouda, Cheddar and Cheshire cheeses.
5. Inhibition of the growth of many species of non-starter bacteria, especially pathogens, food-poisoning and defect-producing micro-organisms.

CHEESE FLAVOUR - AN OVERVIEW

P. F. Fox,
Dept. Food Chemistry,
University College,
Cork, Ireland.

It will be apparent that the metabolism of lactose and lactate in cheese is well understood. In quantitative terms, these changes are among the principal metabolic events in most cheese varieties. However, in comparison with some other biochemical changes during cheese ripening, the conversion of lactose to lactate may have relatively little direct effect on the flavour of mature cheese but since it determines its pH it is of major significance in regulating the various other biochemical reactions that occur during ripening. The isomerization of lactate probably has little impact on cheese flavour, but its conversion to propionate and/or acetate is probably significant and if it does occur, the metabolism of lactate to butyrate has a major negative effect on cheese quality.

Presented at the Eleventh Biennial Cheese Conference, Utah State University,
Logan, Utah. August 16 - 18, 1994.

CONTROLLING CHEESEMAKING: WHY AND HOW

Dr. M. Rosenberg
Dept. of Food Science and Technology
University of California, Davis

Presented and distributed at the
Third California Cheese Symposium
February 14-15, 1994

Controlling the pH

pH has a multitude of effects on cheese properties. It affects the activity of coagulating enzymes, it affects the mineral distribution in milk, it influences the moisture content of cheese, determine the type, rate and extent of biochemical reactions during the ripening and influences and controls the development of different microbial populations in the curd and the cheese. This list makes it clear that pH affects all the quality attributes of cheese, and in many cases, determines the rate at which these characteristics are developed. The effects of pH and its control should be discussed in light of its effects on cheese composition as well as on the activity of all the bio-active components in the system. In some cases, pH manifest synergistic effects with other component of the curd. Such cases are for example the combined effect of pH and salt on microbial activities, combined effect of pH and ionic strength on enzymatic activities etc.

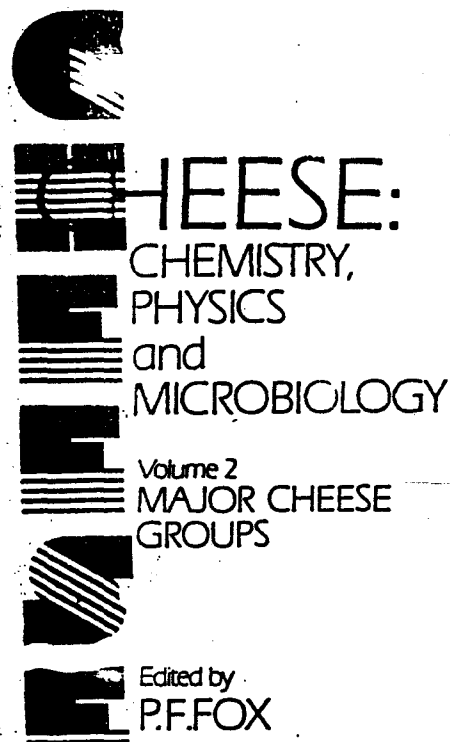
DON'T COMPROMISE QUALITY WITH SHORTCUTS

Mark E. Johnson
Wisconsin Center For Dairy Research

CALCIUM-PHOSPHATE CASEIN COMPLEX

Most of the casein in milk exists in packets called micelles. However, each micelle is actually composed of aggregates of casein molecules called submicelles. There are several forces that keep micelles together but one of the most important is the ionic interactions of the casein with a complex of calcium phosphate. This mineral acts as a sort of glue but it can easily be dissolved by acid or more precisely, a drop in the pH. The calcium phosphate holds the casein molecules together, conceivably in a rather rigid manner preventing or hindering the individual molecules from changing configuration. Unless this rigidity is relaxed, few new interactions are possible. One consequence will be that syneresis, which demands that new interactions occur, will be slowed. On the other hand when the pH drops the calcium phosphate will be dissolved opening up the possibility of new interactions and an increase in the rate of syneresis. However, other fundamental changes are occurring as calcium or calcium phosphate is lost from the protein. Proteins are negatively charged macromolecules. Calcium is a positively charged ion which neutralizes some of the negative charge of the protein. As the calcium is dissolved from the protein, the protein becomes increasingly more negative. Similar to magnets where like charges repel each other, so it is with the negatively charged proteins. The ionic interactions between proteins decrease, resulting in major changes in the physical properties of curd and cheese. At both a high or very low level of protein to protein interaction the cheese will not be flexible or pliable; it will not stretch. However, between the two extremes there is region of just the right amount of protein interaction (or loss of it) that results in the cheese being pliable and where the cheese can easily be stretched. Keep in mind that there are other forces such as hydrophobic interactions that also help keep proteins together.

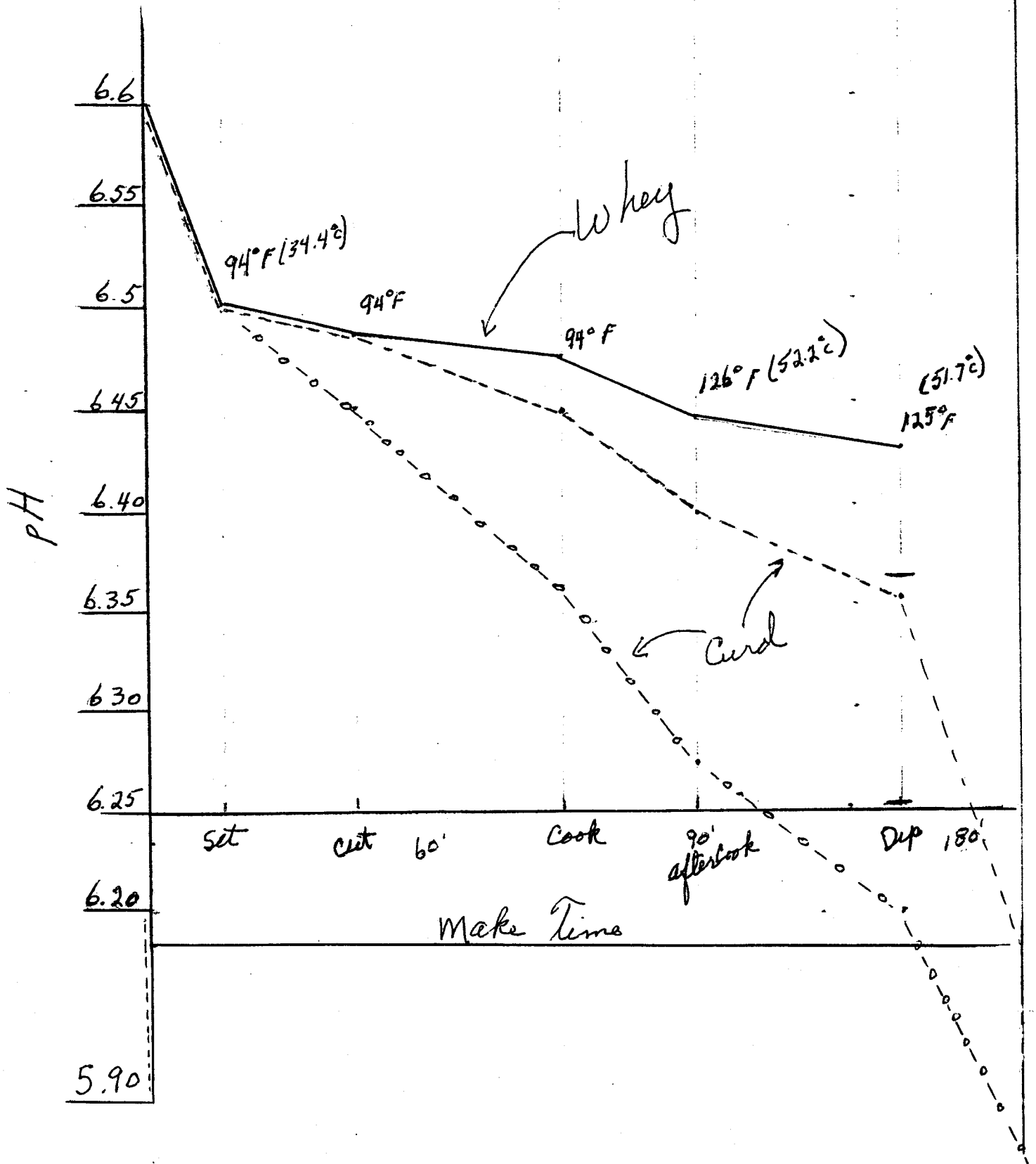
R. C. Lawrence and J. Gilles



The Role of Calcium

The calcium content of Cheddar cheese has an important effect upon both texture and long-term keeping quality.³ Variations in the calcium content occur as a result of differences in the starter percentage used, the time allowed from 'set' to 'run' during manufacture and because of both seasonal and lactational changes. The point in the process at which the curd is drained from the whey is the key stage in the manufacture of Cheddar cheese since it controls to a large extent its mineral content, the proportions of residual chymosin and plasmin in the cheese, the final pH and the moisture to casein ratio.³ All of these influence the rate of proteolysis in the cheese. Furthermore, a relationship has been found between the calcium content of the cheese, the concentration of residual chymosin and the protein breakdown during ripening⁴ and also between the rate of acid development in the early stages of manufacture and the proteolysis in the cheese.^{1,4,5} It follows then that the calcium level is an index of both the extent of acid production up to the draining stage and also an indication of the rate of proteolysis that is likely to occur during ripening. Significant differences in the calcium content of Cheddar cheese manufactured on the same day would suggest differences in the proportions of residual chymosin and plasmin in the cheeses. Consequently, one would expect differences in the rate of proteolysis and thus in the development of flavour.

The introduction of minimum calcium levels into the cheese specifications would further improve the prediction rate when grading a young cheese, since a calcium content above a predetermined level would indicate that acid production up to the draining stage had been normal. It must be stressed, however, that the variations in the calcium content of Cheddar cheese have a much smaller effect than S/M or MNFS on cheese quality⁵ and calcium data should only be used to complement these more important quality parameters. It should also be noted that the routine determination of calcium in cheese presents some problems,^{1,4,6} with a precision of only $\pm 5\%$.



METHOD OF ANALYSIS: QUINHYDRONE - GOLD ELECTRODE

SAMPLE:	1	2	3	4	5	6	7	8	9
lab. 1	5.18	5.24	5.07	5.21	5.12	5.46	5.27	5.48	5.80
2	5.20	5.32	5.15	5.39	5.22	5.51	5.37	5.52	5.74
4	5.07	5.20	5.01	5.22	5.10	5.41	5.22	5.41	5.64
5	5.13	5.26	5.08	5.29	5.18	5.48	5.27	5.48	5.78
7	5.19	5.29	5.12	5.36	5.23	5.54	5.34	5.54	5.76
8	5.15	5.29	5.08	5.25	5.17	5.49	5.30	5.47	5.71
9	5.15	5.24	5.05	5.27	5.14	5.26	5.24	5.37	5.64
11	5.14	5.27	5.09	5.28	5.14	5.42	5.27	5.41	5.68
12	5.04	5.24	5.08	5.29	5.20	5.44	5.30	5.47	5.71
HIGH	5.20	5.32	5.15	5.39	5.23	5.54	5.37	5.54	5.80
LOW	5.04	5.20	5.01	5.21	5.10	5.26	5.22	5.37	5.64

.16 .12 .14 .18 .13 .28 .15 .17 .16

METHOD OF ANALYSIS: POTENTIOMETRIC (Glass bulb/Flat Surface)

SAMPLE:	1	2	3	4	5	6	7	8	9
LAB. 1	5.36	5.41	5.19	5.39	5.27	5.81	5.69	5.49	5.60
2	5.21	5.31	5.22	5.35	5.18	5.41	5.48	5.58	5.78
3	5.07	5.20	5.01	5.22	5.10	5.41	5.22	5.41	5.64
6	5.17	5.33	5.08	5.30	5.18	5.62	5.41	5.48	5.70
7	5.19	5.34	5.10	5.30	5.15	5.50	5.33	5.49	5.73
8	5.12	5.19	4.98	5.14	5.12	5.31	5.23	5.45	5.71
9	5.25	5.27	5.05	5.21	5.11	5.46	5.32	5.45	5.71
10	5.23	5.44	5.20	5.38	5.24	5.59	5.44	5.60	5.85
11	5.20	5.36	5.12	5.31	5.18	5.51	5.35	5.55	5.68
12	5.24	5.37	5.16	5.37	5.24	5.56	5.41	5.55	5.78
HIGH	5.36	5.44	5.22	5.39	5.27	5.81	5.69	5.60	5.85
LOW	5.07	5.19	4.98	5.14	5.10	5.31	5.22	5.41	5.60

.29 .25 .24 .25 .17 .50 .47 .19 .25



6350 North 2150 West
Smithfield, Utah 84335
(801) 563-3281

WESTERN DAIRYMEN COOPERATIVE, INC.

CHEESE DIVISION

The SAM GRAY Gold Electrode™

Highly recommended for accuracy and reliability, this electrode avoids the clogging problems associated with glass electrodes. Most major food processors use it to establish definitive pH readings when buying bulk cheese as a raw material. Plants also use it as a reference for checking accuracy of your glass electrodes.

Used in the gold electrode/quinhydrone method of pH analysis, the SAM GRAY Gold Electrode is referenced in *Standard Methods for the Examination of Dairy Products*, (APHA). This method is ideally suited for high fat, proteinaceous, non-alkaline food products. The primary reasons for this are:

- The reference electrode's junction is isolated from the material being tested;
- The pH electrode does not utilize a glass membrane; and
- The pH measurement utilizes the redox potential of the *entire* sample/quinhydrone mixture, rather than only the ionic potential generated at a glass membrane/sample interface.

As a result: samples cannot clog the electrode; and there are no errors caused by localized pockets of salt, whey or other substances in the sample. Temperature is precisely accounted for in determining pH value. Standard deviation is less than ± 0.02 pH unit.

Each SAM GRAY Gold Electrode is individually checked and assigned a serial number. The electrode has a 99.99% pure gold tip and 40" replaceable lead with strain-relief boot and pin-tip connector. Plastic oversleeve is included for checking electrode accuracy using buffer solutions. Furnished in an attractive wooden box with millivolt/pH conversion tables and certificate of accuracy.

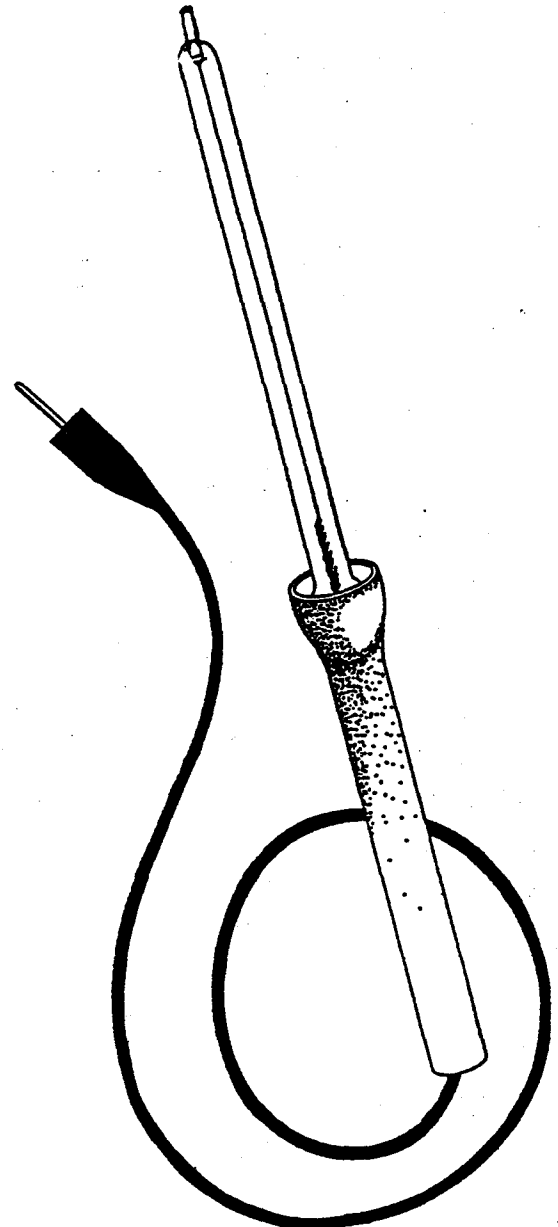
REQUIRED BUT NOT INCLUDED: pH meter with millivolt mode (#140-3297), calomel reference electrode (#140-3573), thermometer (#237-3006), mortar, pestle, beakers, straws, and related items.

Call us for more information about pH testing your food products with the SAM GRAY Gold Electrode!

Stock No.	Description	Sold By
276-3005	SAM GRAY Gold Electrode	EACH

--- RELATED ITEMS ---

Stock No.	Description	Sold By
220-3168	Quinhydrone, 500 gr/jar	JAR
131-3760	KCl solution, 1 qt	BTL
140-3730	Electrode polish, 1 oz	BTL
034-3045	pH 5.0 buffer, 500ml	BTL



NELSON-JAMESON
800/826/8302
FAX 800/472/0840

PH DETERMINATION USING SAM GRAY GOLD ELECTRODE

Available from Nelson-Jameson, Inc.
Marshfield, Wisconsin

CALIBRATION

1. Attach shortening plug, or shortening strap between electrode and reference inputs. Put meter in absolute millivolts. Display should read "-000". If not, put meter into relative millivolts and adjust for display of -.000. This adjusts relative mv to read absolute mv*.
2. Remove shorting plug or shortening strap and insert gold electrode into reference input and calomel cell into pH electrode input.
3. Pour 10-15ml of a buffer solution into beaker. Add quinhydrone (the size of a pea) to the buffer. Mix well. The solution should become dark gray in color.
4. Wet the gold electrode and insert into plastic sleeve. Submerge tip of assembly in quinhydrone buffer solution, and draw solution into sleeve by slowly withdrawing electrode body from sleeve. Invert the electrode assembly and expel any air in the mixture tap to dislodge any bubbles.
5. If necessary repeat step 4 until the gold tip is completely immersed in the mixture, and is midway in the sleeve.
6. Put the gold electrode assembly in the KCL solution with the calomel reference electrode.
7. Record the millivolt reading. Record the temperature of the solution. Using the conversion chart, find pH of the solution to the right of the displayed millivolt reading and under the appropriate temperature.

*Alternate Method Which Permits Correction For System Biases

Use pH/absolute mv conversion chart with a meter in the relative millivolt mode zeroed at pH 7.00.

This permits technicians to offset any bias by "zeroing" instrument using a pH 7 buffer. Steps for pH determination in the relative mode.

1. Zero meter with pH 7 buffer/quinhydrone mixture in sleeve.
2. Note temperature of mixture.
3. Note mv value, for pH 7 buffer at above temperature.
4. Rinse sleeve and electrode.
5. Place electrode tip in straw containing sample quinhydrone mixture and put end of straw in KCL.

6. Note temperature and mv reading for sample.
7. Add mv value for pH 7 buffer to sample reading.
8. Find total on chart UNDER TEMPERATURE FOR SAMPLE. Relate this mv value to pH.

Example: Zero the meter in relative millivolts using a 7.00 buffer at 20 degrees C. Find the absolute millivolt value for this buffer on the conversion chart for 20 degrees C, eg. 46 mv. With the meter still in relative millivolt mode and zeroed to the above buffer, a sample produces a reading of 115 mv at 22 degrees C. Add 46 mv for the buffer to 115 mv for the sample. This total is 161 mv. Find this value on the conversion chart UNDER THE TEMPERATURE (22 degrees C) OF THE SAMPLE/QUINHYDRONE MIXTURE. In this example the sample temperature is 22 degrees C, therefore the pH value is 5.00.

PROCEDURE USING METER IN ABSOLUTE MILLIVOLT MODE:

- A. While preparing the cheese sample, immerse tip of calomel electrode in 25 to 50ml of saturated potassium chloride solution in a 100-ml beaker.
- B. Place approximately 5 grams of cheese at room temperature in a mortar.
- C. Add about 1 gram of quinhydrone powder to the mortar and use the pestle to thoroughly mix with the cheese. Record temperature of the mixture.
- D. Repeatedly press a 1-1/2" section of plastic straw into the cheese-quinhydrone mixture until the straw is firmly packed.
- E. With the plastic sleeve removed from the gold electrode, hold a finger over one end of the straw and gently push the gold tip into the sample mixture at the other end of the straw until tip is completely imbedded.
- F. Put the straw end of the electrode assembly into the beaker with the calomel electrode and saturated potassium chloride solution. The gold electrode should be imbedded so that only a portion of the sample is below surface of the potassium chloride. The end of the gold tip must be above this solution.
- G. Determine and record the millivolt reading (mv) and temperature.
- H. Determine pH value from conversion chart.

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 20.0 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.28	7.27	7.25	7.23	7.22	7.20	7.18	7.16	7.15	7.13
40	7.11	7.10	7.08	7.06	7.04	7.03	7.01	6.99	6.97	6.96
50	6.94	6.92	6.91	6.89	6.87	6.85	6.84	6.82	6.80	6.79
60	6.77	6.75	6.73	6.72	6.70	6.68	6.67	6.65	6.63	6.61
70	6.60	6.58	6.56	6.54	6.53	6.51	6.49	6.48	6.46	6.44
80	6.42	6.41	6.39	6.37	6.36	6.34	6.32	6.30	6.29	6.27
90	6.25	6.23	6.22	6.20	6.18	6.17	6.15	6.13	6.11	6.10
100	6.08	6.06	6.05	6.03	6.01	5.99	5.98	5.96	5.94	5.93
110	5.91	5.89	5.87	5.86	5.84	5.82	5.80	5.79	5.77	5.75
120	5.74	5.72	5.70	5.68	5.67	5.65	5.63	5.62	5.60	5.58
130	5.56	5.55	5.53	5.51	5.49	5.48	5.46	5.44	5.43	5.41
140	5.39	5.37	5.36	5.34	5.32	5.31	5.29	5.27	5.25	5.24
150	5.22	5.20	5.19	5.17	5.15	5.13	5.12	5.10	5.08	5.06
160	5.05	5.03	5.01	5.00	4.98	4.96	4.94	4.93	4.91	4.89
170	4.88	4.86	4.84	4.82	4.81	4.79	4.77	4.75	4.74	4.72
180	4.70	4.69	4.67	4.65	4.63	4.62	4.60	4.58	4.57	4.55
190	4.53	4.51	4.50	4.48	4.46	4.45	4.43	4.41	4.39	4.38
200	4.36	4.34	4.32	4.31	4.29	4.27	4.26	4.24	4.22	4.20
210	4.19	4.17	4.15	4.14	4.12	4.10	4.08	4.07	4.05	4.03
220	4.01	4.00	3.98	3.96	3.95	3.93	3.91	3.89	3.88	3.86

TEMP. 20.5 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.27	7.26	7.24	7.22	7.20	7.19	7.17	7.15	7.13	7.12
40	7.10	7.08	7.07	7.05	7.03	7.01	7.00	6.98	6.96	6.95
50	6.93	6.91	6.89	6.88	6.86	6.84	6.83	6.81	6.79	6.77
60	6.76	6.74	6.72	6.71	6.69	6.67	6.65	6.64	6.62	6.60
70	6.59	6.57	6.55	6.53	6.52	6.50	6.48	6.46	6.45	6.43
80	6.41	6.40	6.38	6.36	6.34	6.33	6.31	6.29	6.28	6.26
90	6.24	6.22	6.21	6.19	6.17	6.16	6.14	6.12	6.10	6.09
100	6.07	6.05	6.04	6.02	6.00	5.98	5.97	5.95	5.93	5.92
110	5.90	5.88	5.86	5.85	5.83	5.81	5.79	5.78	5.76	5.74
120	5.73	5.71	5.69	5.67	5.66	5.64	5.62	5.61	5.59	5.57
130	5.55	5.54	5.52	5.50	5.49	5.47	5.45	5.43	5.42	5.40
140	5.38	5.37	5.35	5.33	5.31	5.30	5.28	5.26	5.25	5.23
150	5.21	5.19	5.18	5.16	5.14	5.12	5.11	5.09	5.07	5.06
160	5.04	5.02	5.00	4.99	4.97	4.95	4.94	4.92	4.90	4.88
170	4.87	4.85	4.83	4.82	4.80	4.78	4.76	4.75	4.73	4.71
180	4.70	4.68	4.66	4.64	4.63	4.61	4.59	4.58	4.56	4.54
190	4.52	4.51	4.49	4.47	4.45	4.44	4.42	4.40	4.39	4.37
200	4.35	4.33	4.32	4.30	4.28	4.27	4.25	4.23	4.21	4.20
210	4.18	4.16	4.15	4.13	4.11	4.09	4.08	4.06	4.04	4.03
220	4.01	3.99	3.97	3.96	3.94	3.92	3.91	3.89	3.87	3.85

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 21.0 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.26	7.24	7.23	7.21	7.19	7.17	7.16	7.14	7.12	7.11
40	7.09	7.07	7.05	7.04	7.02	7.00	6.99	6.97	6.95	6.93
50	6.92	6.90	6.88	6.87	6.85	6.83	6.81	6.80	6.78	6.76
60	6.75	6.73	6.71	6.69	6.68	6.66	6.64	6.63	6.61	6.59
70	6.57	6.56	6.54	6.52	6.51	6.49	6.47	6.45	6.44	6.42
80	6.40	6.39	6.37	6.35	6.33	6.32	6.30	6.28	6.27	6.25
90	6.23	6.21	6.20	6.18	6.16	6.15	6.13	6.11	6.09	6.08
100	6.06	6.04	6.03	6.01	5.99	5.97	5.96	5.94	5.92	5.91
110	5.89	5.87	5.85	5.84	5.82	5.80	5.79	5.77	5.75	5.73
120	5.72	5.70	5.68	5.67	5.65	5.63	5.61	5.60	5.58	5.56
130	5.55	5.53	5.51	5.49	5.48	5.46	5.44	5.43	5.41	5.39
140	5.37	5.36	5.34	5.32	5.31	5.29	5.27	5.25	5.24	5.22
150	5.20	5.18	5.17	5.15	5.13	5.12	5.10	5.08	5.06	5.05
160	5.03	5.01	5.00	4.98	4.96	4.94	4.93	4.91	4.89	4.88
170	4.86	4.84	4.82	4.81	4.79	4.77	4.76	4.74	4.72	4.70
180	4.69	4.67	4.65	4.64	4.62	4.60	4.58	4.57	4.55	4.53
190	4.52	4.50	4.48	4.46	4.45	4.43	4.41	4.40	4.38	4.36
200	4.34	4.33	4.31	4.29	4.28	4.26	4.24	4.22	4.21	4.19
210	4.17	4.16	4.14	4.12	4.10	4.09	4.07	4.05	4.04	4.02
220	4.00	3.98	3.97	3.95	3.93	3.92	3.90	3.88	3.86	3.85

TEMP. 21.5 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.25	7.23	7.21	7.20	7.18	7.16	7.15	7.13	7.11	7.09
40	7.08	7.06	7.04	7.03	7.01	6.99	6.97	6.96	6.94	6.92
50	6.91	6.89	6.87	6.85	6.84	6.82	6.80	6.79	6.77	6.75
60	6.73	6.72	6.70	6.68	6.67	6.65	6.63	6.61	6.60	6.58
70	6.56	6.55	6.53	6.51	6.49	6.48	6.46	6.44	6.43	6.41
80	6.39	6.37	6.36	6.34	6.32	6.31	6.29	6.27	6.26	6.24
90	6.22	6.20	6.19	6.17	6.15	6.14	6.12	6.10	6.08	6.07
100	6.05	6.03	6.02	6.00	5.98	5.96	5.95	5.93	5.91	5.90
110	5.88	5.86	5.84	5.83	5.81	5.79	5.78	5.76	5.74	5.72
120	5.71	5.69	5.67	5.66	5.64	5.62	5.60	5.59	5.57	5.55
130	5.54	5.52	5.50	5.48	5.47	5.45	5.43	5.42	5.40	5.38
140	5.36	5.35	5.33	5.31	5.30	5.28	5.26	5.24	5.23	5.21
150	5.19	5.18	5.16	5.14	5.13	5.11	5.09	5.07	5.06	5.04
160	5.02	5.01	4.99	4.97	4.95	4.94	4.92	4.90	4.89	4.87
170	4.85	4.83	4.82	4.80	4.78	4.77	4.75	4.73	4.71	4.70
180	4.68	4.66	4.65	4.63	4.61	4.59	4.58	4.56	4.54	4.53
190	4.51	4.49	4.47	4.46	4.44	4.42	4.41	4.39	4.37	4.35
200	4.34	4.32	4.30	4.29	4.27	4.25	4.23	4.22	4.20	4.18
210	4.17	4.15	4.13	4.11	4.10	4.08	4.06	4.05	4.03	4.01
220	3.99	3.98	3.96	3.94	3.93	3.91	3.89	3.88	3.86	3.84

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 22.0 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.24	7.22	7.20	7.18	7.17	7.15	7.13	7.12	7.10	7.08
40	7.07	7.05	7.03	7.01	7.00	6.98	6.96	6.95	6.93	6.91
50	6.89	6.88	6.86	6.84	6.83	6.81	6.79	6.77	6.76	6.74
60	6.72	6.71	6.69	6.67	6.65	6.64	6.62	6.60	6.59	6.57
70	6.55	6.54	6.52	6.50	6.48	6.47	6.45	6.43	6.42	6.40
80	6.38	6.36	6.35	6.33	6.31	6.30	6.28	6.26	6.24	6.23
90	6.21	6.19	6.18	6.16	6.14	6.12	6.11	6.09	6.07	6.06
100	6.04	6.02	6.01	5.99	5.97	5.95	5.94	5.92	5.90	5.89
110	5.87	5.85	5.83	5.82	5.80	5.78	5.77	5.75	5.73	5.71
120	5.70	5.68	5.66	5.65	5.63	5.61	5.60	5.58	5.56	5.54
130	5.53	5.51	5.49	5.48	5.46	5.44	5.42	5.41	5.39	5.37
140	5.36	5.34	5.32	5.30	5.29	5.27	5.25	5.24	5.22	5.20
150	5.18	5.17	5.15	5.13	5.12	5.10	5.08	5.07	5.05	5.03
160	5.01	5.00	4.98	4.96	4.95	4.93	4.91	4.89	4.88	4.86
170	4.84	4.83	4.81	4.79	4.77	4.76	4.74	4.72	4.71	4.69
180	4.67	4.65	4.64	4.62	4.60	4.59	4.57	4.55	4.54	4.52
190	4.50	4.48	4.47	4.45	4.43	4.42	4.40	4.38	4.36	4.35
200	4.33	4.31	4.30	4.28	4.26	4.24	4.23	4.21	4.19	4.18
210	4.16	4.14	4.13	4.11	4.09	4.07	4.06	4.04	4.02	4.01
220	3.99	3.97	3.95	3.94	3.92	3.90	3.89	3.87	3.85	3.83

TEMP. 22.5 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.22	7.21	7.19	7.17	7.16	7.14	7.12	7.10	7.09	7.07
40	7.05	7.04	7.02	7.00	6.99	6.97	6.95	6.93	6.92	6.90
50	6.88	6.87	6.85	6.83	6.81	6.80	6.78	6.76	6.75	6.73
60	6.71	6.69	6.68	6.66	6.64	6.63	6.61	6.59	6.58	6.56
70	6.54	6.52	6.51	6.49	6.47	6.46	6.44	6.42	6.40	6.39
80	6.37	6.35	6.34	6.32	6.30	6.29	6.27	6.25	6.23	6.22
90	6.20	6.18	6.17	6.15	6.13	6.11	6.10	6.08	6.06	6.05
100	6.03	6.01	6.00	5.98	5.96	5.94	5.93	5.91	5.89	5.88
110	5.86	5.84	5.82	5.81	5.79	5.77	5.76	5.74	5.72	5.71
120	5.69	5.67	5.65	5.64	5.62	5.60	5.59	5.57	5.55	5.53
130	5.52	5.50	5.48	5.47	5.45	5.43	5.42	5.40	5.38	5.36
140	5.35	5.33	5.31	5.30	5.28	5.26	5.24	5.23	5.21	5.19
150	5.18	5.16	5.14	5.13	5.11	5.09	5.07	5.06	5.04	5.02
160	5.01	4.99	4.97	4.95	4.94	4.92	4.90	4.89	4.87	4.85
170	4.83	4.82	4.80	4.78	4.77	4.75	4.73	4.72	4.70	4.68
180	4.66	4.65	4.63	4.61	4.60	4.58	4.56	4.54	4.53	4.51
190	4.49	4.48	4.46	4.44	4.43	4.41	4.39	4.37	4.36	4.34
200	4.32	4.31	4.29	4.27	4.25	4.24	4.22	4.20	4.19	4.17
210	4.15	4.14	4.12	4.10	4.08	4.07	4.05	4.03	4.02	4.00
220	3.98	3.96	3.95	3.93	3.91	3.90	3.88	3.86	3.85	3.83

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 23.0 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.21	7.19	7.18	7.16	7.14	7.13	7.11	7.09	7.08	7.06
40	7.04	7.02	7.01	6.99	6.97	6.96	6.94	6.92	6.91	6.89
50	6.87	6.85	6.84	6.82	6.80	6.79	6.77	6.75	6.73	6.72
60	6.70	6.68	6.67	6.65	6.63	6.62	6.60	6.58	6.56	6.55
70	6.53	6.51	6.50	6.48	6.46	6.45	6.43	6.41	6.39	6.38
80	6.36	6.34	6.33	6.31	6.29	6.27	6.26	6.24	6.22	6.21
90	6.19	6.17	6.16	6.14	6.12	6.10	6.09	6.07	6.05	6.04
100	6.02	6.00	5.99	5.97	5.95	5.93	5.92	5.90	5.88	5.87
110	5.85	5.83	5.82	5.80	5.78	5.76	5.75	5.73	5.71	5.70
120	5.68	5.66	5.64	5.63	5.61	5.59	5.58	5.56	5.54	5.53
130	5.51	5.49	5.47	5.46	5.44	5.42	5.41	5.39	5.37	5.36
140	5.34	5.32	5.30	5.29	5.27	5.25	5.24	5.22	5.20	5.18
150	5.17	5.15	5.13	5.12	5.10	5.08	5.07	5.05	5.03	5.01
160	5.00	4.98	4.96	4.95	4.93	4.91	4.90	4.88	4.86	4.84
170	4.83	4.81	4.79	4.78	4.76	4.74	4.72	4.71	4.69	4.67
180	4.66	4.64	4.62	4.61	4.59	4.57	4.55	4.54	4.52	4.50
190	4.49	4.47	4.45	4.44	4.42	4.40	4.38	4.37	4.35	4.33
200	4.32	4.30	4.28	4.26	4.25	4.23	4.21	4.20	4.18	4.16
210	4.15	4.13	4.11	4.09	4.08	4.06	4.04	4.03	4.01	3.99
220	3.98	3.96	3.94	3.92	3.91	3.89	3.87	3.86	3.84	3.82

TEMP. 23.5 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.20	7.18	7.17	7.15	7.13	7.11	7.10	7.08	7.06	7.05
40	7.03	7.01	7.00	6.98	6.96	6.94	6.93	6.91	6.89	6.88
50	6.86	6.84	6.83	6.81	6.79	6.77	6.76	6.74	6.72	6.71
60	6.69	6.67	6.66	6.64	6.62	6.60	6.59	6.57	6.55	6.54
70	6.52	6.50	6.49	6.47	6.45	6.43	6.42	6.40	6.38	6.37
80	6.35	6.33	6.32	6.30	6.28	6.26	6.25	6.23	6.21	6.20
90	6.18	6.16	6.15	6.13	6.11	6.09	6.08	6.06	6.04	6.03
100	6.01	5.99	5.98	5.96	5.94	5.92	5.91	5.89	5.87	5.86
110	5.84	5.82	5.81	5.79	5.77	5.75	5.74	5.72	5.70	5.69
120	5.67	5.65	5.64	5.62	5.60	5.58	5.57	5.55	5.53	5.52
130	5.50	5.48	5.47	5.45	5.43	5.41	5.40	5.38	5.36	5.35
140	5.33	5.31	5.30	5.28	5.26	5.24	5.23	5.21	5.19	5.18
150	5.16	5.14	5.13	5.11	5.09	5.07	5.06	5.04	5.02	5.01
160	4.99	4.97	4.96	4.94	4.92	4.90	4.89	4.87	4.85	4.84
170	4.82	4.80	4.79	4.77	4.75	4.73	4.72	4.70	4.68	4.67
180	4.65	4.63	4.61	4.60	4.58	4.56	4.55	4.53	4.51	4.50
190	4.48	4.46	4.44	4.43	4.41	4.39	4.38	4.36	4.34	4.33
200	4.31	4.29	4.27	4.26	4.24	4.22	4.21	4.19	4.17	4.16
210	4.14	4.12	4.10	4.09	4.07	4.05	4.04	4.02	4.00	3.99
220	3.97	3.95	3.93	3.92	3.90	3.88	3.87	3.85	3.83	3.82

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 24.0 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.19	7.17	7.15	7.14	7.12	7.10	7.09	7.07	7.05	7.04
40	7.02	7.00	6.98	6.97	6.95	6.93	6.92	6.90	6.88	6.87
50	6.85	6.83	6.81	6.80	6.78	6.76	6.75	6.73	6.71	6.70
60	6.68	6.66	6.64	6.63	6.61	6.59	6.58	6.56	6.54	6.53
70	6.51	6.49	6.47	6.46	6.44	6.42	6.41	6.39	6.37	6.36
80	6.34	6.32	6.31	6.29	6.27	6.25	6.24	6.22	6.20	6.19
90	6.17	6.15	6.14	6.12	6.10	6.08	6.07	6.05	6.03	6.02
100	6.00	5.98	5.97	5.95	5.93	5.91	5.90	5.88	5.86	5.85
110	5.83	5.81	5.80	5.78	5.76	5.74	5.73	5.71	5.69	5.68
120	5.66	5.64	5.63	5.61	5.59	5.58	5.56	5.54	5.52	5.51
130	5.49	5.47	5.46	5.44	5.42	5.41	5.39	5.37	5.35	5.34
140	5.32	5.30	5.29	5.27	5.25	5.24	5.22	5.20	5.18	5.17
150	5.15	5.13	5.12	5.10	5.08	5.07	5.05	5.03	5.01	5.00
160	4.98	4.96	4.95	4.93	4.91	4.90	4.88	4.86	4.85	4.83
170	4.81	4.79	4.78	4.76	4.74	4.73	4.71	4.69	4.68	4.66
180	4.64	4.62	4.61	4.59	4.57	4.56	4.54	4.52	4.51	4.49
190	4.47	4.45	4.44	4.42	4.40	4.39	4.37	4.35	4.34	4.32
200	4.30	4.28	4.27	4.25	4.23	4.22	4.20	4.18	4.17	4.15
210	4.13	4.11	4.10	4.08	4.06	4.05	4.03	4.01	4.00	3.98
220	3.96	3.95	3.93	3.91	3.89	3.88	3.86	3.84	3.83	3.81

TEMP. 24.5 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.18	7.16	7.14	7.13	7.11	7.09	7.07	7.06	7.04	7.02
40	7.01	6.99	6.97	6.96	6.94	6.92	6.90	6.89	6.87	6.85
50	6.84	6.82	6.80	6.79	6.77	6.75	6.74	6.72	6.70	6.68
60	6.67	6.65	6.63	6.62	6.60	6.58	6.57	6.55	6.53	6.51
70	6.50	6.48	6.46	6.45	6.43	6.41	6.40	6.38	6.36	6.35
80	6.33	6.31	6.29	6.28	6.26	6.24	6.23	6.21	6.19	6.18
90	6.16	6.14	6.13	6.11	6.09	6.07	6.06	6.04	6.02	6.01
100	5.99	5.97	5.96	5.94	5.92	5.90	5.89	5.87	5.85	5.84
110	5.82	5.80	5.79	5.77	5.75	5.74	5.72	5.70	5.68	5.67
120	5.65	5.63	5.62	5.60	5.58	5.57	5.55	5.53	5.52	5.50
130	5.48	5.46	5.45	5.43	5.41	5.40	5.38	5.36	5.35	5.33
140	5.31	5.29	5.28	5.26	5.24	5.23	5.21	5.19	5.18	5.16
150	5.14	5.13	5.11	5.09	5.07	5.06	5.04	5.02	5.01	4.99
160	4.97	4.96	4.94	4.92	4.90	4.89	4.87	4.85	4.84	4.82
170	4.80	4.79	4.77	4.75	4.74	4.72	4.70	4.68	4.67	4.65
180	4.63	4.62	4.60	4.58	4.57	4.55	4.53	4.52	4.50	4.48
190	4.46	4.45	4.43	4.41	4.40	4.38	4.36	4.35	4.33	4.31
200	4.29	4.28	4.26	4.24	4.23	4.21	4.19	4.18	4.16	4.14
210	4.13	4.11	4.09	4.07	4.06	4.04	4.02	4.01	3.99	3.97
220	3.96	3.94	3.92	3.90	3.89	3.87	3.85	3.84	3.82	3.80

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 25.0 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.16	7.15	7.13	7.11	7.10	7.08	7.06	7.05	7.03	7.01
40	6.99	6.98	6.96	6.94	6.93	6.91	6.89	6.88	6.86	6.84
50	6.83	6.81	6.79	6.77	6.76	6.74	6.72	6.71	6.69	6.67
60	6.66	6.64	6.62	6.61	6.59	6.57	6.55	6.54	6.52	6.50
70	6.49	6.47	6.45	6.44	6.42	6.40	6.39	6.37	6.35	6.34
80	6.32	6.30	6.28	6.27	6.25	6.23	6.22	6.20	6.18	6.17
90	6.15	6.13	6.12	6.10	6.08	6.06	6.05	6.03	6.01	6.00
100	5.98	5.96	5.95	5.93	5.91	5.90	5.88	5.86	5.84	5.83
110	5.81	5.79	5.78	5.76	5.74	5.73	5.71	5.69	5.68	5.66
120	5.64	5.62	5.61	5.59	5.57	5.56	5.54	5.52	5.51	5.49
130	5.47	5.46	5.44	5.42	5.40	5.39	5.37	5.35	5.34	5.32
140	5.30	5.29	5.27	5.25	5.24	5.22	5.20	5.18	5.17	5.15
150	5.13	5.12	5.10	5.08	5.07	5.05	5.03	5.02	5.00	4.98
160	4.96	4.95	4.93	4.91	4.90	4.88	4.86	4.85	4.83	4.81
170	4.80	4.78	4.76	4.74	4.73	4.71	4.69	4.68	4.66	4.64
180	4.63	4.61	4.59	4.58	4.56	4.54	4.52	4.51	4.49	4.47
190	4.46	4.44	4.42	4.41	4.39	4.37	4.36	4.34	4.32	4.30
200	4.29	4.27	4.25	4.24	4.22	4.20	4.19	4.17	4.15	4.14
210	4.12	4.10	4.08	4.07	4.05	4.03	4.02	4.00	3.98	3.97
220	3.95	3.93	3.92	3.90	3.88	3.86	3.85	3.83	3.81	3.80

TEMP. 25.5 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.15	7.14	7.12	7.10	7.08	7.07	7.05	7.03	7.02	7.00
40	6.98	6.97	6.95	6.93	6.92	6.90	6.88	6.87	6.85	6.83
50	6.81	6.80	6.78	6.76	6.75	6.73	6.71	6.70	6.68	6.66
60	6.65	6.63	6.61	6.59	6.58	6.56	6.54	6.53	6.51	6.49
70	6.48	6.46	6.44	6.43	6.41	6.39	6.38	6.36	6.34	6.32
80	6.31	6.29	6.27	6.26	6.24	6.22	6.21	6.19	6.17	6.16
90	6.14	6.12	6.10	6.09	6.07	6.05	6.04	6.02	6.00	5.99
100	5.97	5.95	5.94	5.92	5.90	5.89	5.87	5.85	5.83	5.82
110	5.80	5.78	5.77	5.75	5.73	5.72	5.70	5.68	5.67	5.65
120	5.63	5.62	5.60	5.58	5.56	5.55	5.53	5.51	5.50	5.48
130	5.46	5.45	5.43	5.41	5.40	5.38	5.36	5.34	5.33	5.31
140	5.29	5.28	5.26	5.24	5.23	5.21	5.19	5.18	5.16	5.14
150	5.13	5.11	5.09	5.07	5.06	5.04	5.02	5.01	4.99	4.97
160	4.96	4.94	4.92	4.91	4.89	4.87	4.85	4.84	4.82	4.80
170	4.79	4.77	4.75	4.74	4.72	4.70	4.69	4.67	4.65	4.64
180	4.62	4.60	4.58	4.57	4.55	4.53	4.52	4.50	4.48	4.47
190	4.45	4.43	4.42	4.40	4.38	4.37	4.35	4.33	4.31	4.30
200	4.28	4.26	4.25	4.23	4.21	4.20	4.18	4.16	4.15	4.13
210	4.11	4.09	4.08	4.06	4.04	4.03	4.01	3.99	3.98	3.96
220	3.94	3.93	3.91	3.89	3.88	3.86	3.84	3.82	3.81	3.79

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 26.0 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.14	7.12	7.11	7.09	7.07	7.06	7.04	7.02	7.01	6.99
40	6.97	6.96	6.94	6.92	6.90	6.89	6.87	6.85	6.84	6.82
50	6.80	6.79	6.77	6.75	6.74	6.72	6.70	6.69	6.67	6.65
60	6.63	6.62	6.60	6.58	6.57	6.55	6.53	6.52	6.50	6.48
70	6.47	6.45	6.43	6.42	6.40	6.38	6.36	6.35	6.33	6.31
80	6.30	6.28	6.26	6.25	6.23	6.21	6.20	6.18	6.16	6.15
90	6.13	6.11	6.09	6.08	6.06	6.04	6.03	6.01	5.99	5.98
100	5.96	5.94	5.93	5.91	5.89	5.88	5.86	5.84	5.83	5.81
110	5.79	5.77	5.76	5.74	5.72	5.71	5.69	5.67	5.66	5.64
120	5.62	5.61	5.59	5.57	5.56	5.54	5.52	5.50	5.49	5.47
130	5.45	5.44	5.42	5.40	5.39	5.37	5.35	5.34	5.32	5.30
140	5.29	5.27	5.25	5.23	5.22	5.20	5.18	5.17	5.15	5.13
150	5.12	5.10	5.08	5.07	5.05	5.03	5.02	5.00	4.98	4.97
160	4.95	4.93	4.91	4.90	4.88	4.86	4.85	4.83	4.81	4.80
170	4.78	4.76	4.75	4.73	4.71	4.70	4.68	4.66	4.64	4.63
180	4.61	4.59	4.58	4.56	4.54	4.53	4.51	4.49	4.48	4.46
190	4.44	4.43	4.41	4.39	4.37	4.36	4.34	4.32	4.31	4.29
200	4.27	4.26	4.24	4.22	4.21	4.19	4.17	4.16	4.14	4.12
210	4.11	4.09	4.07	4.05	4.04	4.02	4.00	3.99	3.97	3.95
220	3.94	3.92	3.90	3.89	3.87	3.85	3.84	3.82	3.80	3.78

TEMP. 26.5 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.13	7.11	7.10	7.08	7.06	7.04	7.03	7.01	6.99	6.98
40	6.96	6.94	6.93	6.91	6.89	6.88	6.86	6.84	6.83	6.81
50	6.79	6.78	6.76	6.74	6.72	6.71	6.69	6.67	6.66	6.64
60	6.62	6.61	6.59	6.57	6.56	6.54	6.52	6.51	6.49	6.47
70	6.46	6.44	6.42	6.40	6.39	6.37	6.35	6.34	6.32	6.30
80	6.29	6.27	6.25	6.24	6.22	6.20	6.19	6.17	6.15	6.14
90	6.12	6.10	6.08	6.07	6.05	6.03	6.02	6.00	5.98	5.97
100	5.95	5.93	5.92	5.90	5.88	5.87	5.85	5.83	5.82	5.80
110	5.78	5.77	5.75	5.73	5.71	5.70	5.68	5.66	5.65	5.63
120	5.61	5.60	5.58	5.56	5.55	5.53	5.51	5.50	5.48	5.46
130	5.45	5.43	5.41	5.39	5.38	5.36	5.34	5.33	5.31	5.29
140	5.28	5.26	5.24	5.23	5.21	5.19	5.18	5.16	5.14	5.13
150	5.11	5.09	5.07	5.06	5.04	5.02	5.01	4.99	4.97	4.96
160	4.94	4.92	4.91	4.89	4.87	4.86	4.84	4.82	4.81	4.79
170	4.77	4.75	4.74	4.72	4.70	4.69	4.67	4.65	4.64	4.62
180	4.60	4.59	4.57	4.55	4.54	4.52	4.50	4.49	4.47	4.45
190	4.44	4.42	4.40	4.38	4.37	4.35	4.33	4.32	4.30	4.28
200	4.27	4.25	4.23	4.22	4.20	4.18	4.17	4.15	4.13	4.12
210	4.10	4.08	4.06	4.05	4.03	4.01	4.00	3.98	3.96	3.95
220	3.93	3.91	3.90	3.88	3.86	3.85	3.83	3.81	3.80	3.78

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 27.0 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.12	7.10	7.08	7.07	7.05	7.03	7.02	7.00	6.98	6.97
40	6.95	6.93	6.92	6.90	6.88	6.86	6.85	6.83	6.81	6.80
50	6.78	6.76	6.75	6.73	6.71	6.70	6.68	6.66	6.65	6.63
60	6.61	6.60	6.58	6.56	6.55	6.53	6.51	6.50	6.48	6.46
70	6.44	6.43	6.41	6.39	6.38	6.36	6.34	6.33	6.31	6.29
80	6.28	6.26	6.24	6.23	6.21	6.19	6.18	6.16	6.14	6.13
90	6.11	6.09	6.07	6.06	6.04	6.02	6.01	5.99	5.97	5.96
100	5.94	5.92	5.91	5.89	5.87	5.86	5.84	5.82	5.81	5.79
110	5.77	5.76	5.74	5.72	5.71	5.69	5.67	5.65	5.64	5.62
120	5.60	5.59	5.57	5.55	5.54	5.52	5.50	5.49	5.47	5.45
130	5.44	5.42	5.40	5.39	5.37	5.35	5.34	5.32	5.30	5.29
140	5.27	5.25	5.23	5.22	5.20	5.18	5.17	5.15	5.13	5.12
150	5.10	5.08	5.07	5.05	5.03	5.02	5.00	4.98	4.97	4.95
160	4.93	4.92	4.90	4.88	4.86	4.85	4.83	4.81	4.80	4.78
170	4.76	4.75	4.73	4.71	4.70	4.68	4.66	4.65	4.63	4.61
180	4.60	4.58	4.56	4.55	4.53	4.51	4.50	4.48	4.46	4.44
190	4.43	4.41	4.39	4.38	4.36	4.34	4.33	4.31	4.29	4.28
200	4.26	4.24	4.23	4.21	4.19	4.18	4.16	4.14	4.13	4.11
210	4.09	4.07	4.06	4.04	4.02	4.01	3.99	3.97	3.96	3.94
220	3.92	3.91	3.89	3.87	3.86	3.84	3.82	3.81	3.79	3.77

TEMP. 27.5 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.11	7.09	7.07	7.06	7.04	7.02	7.00	6.99	6.97	6.95
40	6.94	6.92	6.90	6.89	6.87	6.85	6.84	6.82	6.80	6.79
50	6.77	6.75	6.74	6.72	6.70	6.69	6.67	6.65	6.64	6.62
60	6.60	6.59	6.57	6.55	6.53	6.52	6.50	6.48	6.47	6.45
70	6.43	6.42	6.40	6.38	6.37	6.35	6.33	6.32	6.30	6.28
80	6.27	6.25	6.23	6.22	6.20	6.18	6.17	6.15	6.13	6.12
90	6.10	6.08	6.07	6.05	6.03	6.01	6.00	5.98	5.96	5.95
100	5.93	5.91	5.90	5.88	5.86	5.85	5.83	5.81	5.80	5.78
110	5.76	5.75	5.73	5.71	5.70	5.68	5.66	5.65	5.63	5.61
120	5.60	5.58	5.56	5.54	5.53	5.51	5.49	5.48	5.46	5.44
130	5.43	5.41	5.39	5.38	5.36	5.34	5.33	5.31	5.29	5.28
140	5.26	5.24	5.23	5.21	5.19	5.18	5.16	5.14	5.13	5.11
150	5.09	5.08	5.06	5.04	5.02	5.01	4.99	4.97	4.96	4.94
160	4.92	4.91	4.89	4.87	4.86	4.84	4.82	4.81	4.79	4.77
170	4.76	4.74	4.72	4.71	4.69	4.67	4.66	4.64	4.62	4.61
180	4.59	4.57	4.55	4.54	4.52	4.50	4.49	4.47	4.45	4.44
190	4.42	4.40	4.39	4.37	4.35	4.34	4.32	4.30	4.29	4.27
200	4.25	4.24	4.22	4.20	4.19	4.17	4.15	4.14	4.12	4.10
210	4.09	4.07	4.05	4.03	4.02	4.00	3.98	3.97	3.95	3.93
220	3.92	3.90	3.88	3.87	3.85	3.83	3.82	3.80	3.78	3.77

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 28.0 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.09	7.08	7.06	7.04	7.03	7.01	6.99	6.98	6.96	6.94
40	6.93	6.91	6.89	6.88	6.86	6.84	6.83	6.81	6.79	6.78
50	6.76	6.74	6.73	6.71	6.69	6.67	6.66	6.64	6.62	6.61
60	6.59	6.57	6.56	6.54	6.52	6.51	6.49	6.47	6.46	6.44
70	6.42	6.41	6.39	6.37	6.36	6.34	6.32	6.31	6.29	6.27
80	6.26	6.24	6.22	6.21	6.19	6.17	6.16	6.14	6.12	6.11
90	6.09	6.07	6.06	6.04	6.02	6.00	5.99	5.97	5.95	5.94
100	5.92	5.90	5.89	5.87	5.85	5.84	5.82	5.80	5.79	5.77
110	5.75	5.74	5.72	5.70	5.69	5.67	5.65	5.64	5.62	5.60
120	5.59	5.57	5.55	5.54	5.52	5.50	5.49	5.47	5.45	5.44
130	5.42	5.40	5.39	5.37	5.35	5.33	5.32	5.30	5.28	5.27
140	5.25	5.23	5.22	5.20	5.18	5.17	5.15	5.13	5.12	5.10
150	5.08	5.07	5.05	5.03	5.02	5.00	4.98	4.97	4.95	4.93
160	4.92	4.90	4.88	4.87	4.85	4.83	4.82	4.80	4.78	4.77
170	4.75	4.73	4.71	4.70	4.68	4.66	4.65	4.63	4.61	4.60
180	4.58	4.56	4.55	4.53	4.51	4.50	4.48	4.46	4.45	4.43
190	4.41	4.40	4.38	4.36	4.35	4.33	4.31	4.30	4.28	4.26
200	4.25	4.23	4.21	4.20	4.18	4.16	4.15	4.13	4.11	4.10
210	4.08	4.06	4.04	4.03	4.01	3.99	3.98	3.96	3.94	3.93
220	3.91	3.89	3.88	3.86	3.84	3.83	3.81	3.79	3.78	3.76

TEMP. 28.5 C

Absolute

Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.08	7.07	7.05	7.03	7.02	7.00	6.98	6.97	6.95	6.93
40	6.91	6.90	6.88	6.86	6.85	6.83	6.81	6.80	6.78	6.76
50	6.75	6.73	6.71	6.70	6.68	6.66	6.65	6.63	6.61	6.60
60	6.58	6.56	6.55	6.53	6.51	6.50	6.48	6.46	6.45	6.43
70	6.41	6.40	6.38	6.36	6.35	6.33	6.31	6.30	6.28	6.26
80	6.25	6.23	6.21	6.20	6.18	6.16	6.15	6.13	6.11	6.10
90	6.08	6.06	6.05	6.03	6.01	6.00	5.98	5.96	5.94	5.93
100	5.91	5.89	5.88	5.86	5.84	5.83	5.81	5.79	5.78	5.76
110	5.74	5.73	5.71	5.69	5.68	5.66	5.64	5.63	5.61	5.59
120	5.58	5.56	5.54	5.53	5.51	5.49	5.48	5.46	5.44	5.43
130	5.41	5.39	5.38	5.36	5.34	5.33	5.31	5.29	5.28	5.26
140	5.24	5.23	5.21	5.19	5.18	5.16	5.14	5.13	5.11	5.09
150	5.08	5.06	5.04	5.03	5.01	4.99	4.97	4.96	4.94	4.92
160	4.91	4.89	4.87	4.86	4.84	4.82	4.81	4.79	4.77	4.76
170	4.74	4.72	4.71	4.69	4.67	4.66	4.64	4.62	4.61	4.59
180	4.57	4.56	4.54	4.52	4.51	4.49	4.47	4.46	4.44	4.42
190	4.41	4.39	4.37	4.36	4.34	4.32	4.31	4.29	4.27	4.26
200	4.24	4.22	4.21	4.19	4.17	4.16	4.14	4.12	4.11	4.09
210	4.07	4.06	4.04	4.02	4.00	3.99	3.97	3.95	3.94	3.92
220	3.90	3.89	3.87	3.85	3.84	3.82	3.80	3.79	3.77	3.75

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

TEMP. 29.0 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.07	7.05	7.04	7.02	7.00	6.99	6.97	6.95	6.94	6.92
40	6.90	6.89	6.87	6.85	6.84	6.82	6.80	6.79	6.77	6.75
50	6.74	6.72	6.70	6.69	6.67	6.65	6.64	6.62	6.60	6.59
60	6.57	6.55	6.54	6.52	6.50	6.49	6.47	6.45	6.44	6.42
70	6.40	6.39	6.37	6.35	6.34	6.32	6.30	6.29	6.27	6.25
80	6.24	6.22	6.20	6.19	6.17	6.15	6.14	6.12	6.10	6.09
90	6.07	6.05	6.04	6.02	6.00	5.99	5.97	5.95	5.94	5.92
100	5.90	5.89	5.87	5.85	5.84	5.82	5.80	5.78	5.77	5.75
110	5.73	5.72	5.70	5.68	5.67	5.65	5.63	5.62	5.60	5.58
120	5.57	5.55	5.53	5.52	5.50	5.48	5.47	5.45	5.43	5.42
130	5.40	5.38	5.37	5.35	5.33	5.32	5.30	5.28	5.27	5.25
140	5.23	5.22	5.20	5.18	5.17	5.15	5.13	5.12	5.10	5.08
150	5.07	5.05	5.03	5.02	5.00	4.98	4.97	4.95	4.93	4.92
160	4.90	4.88	4.87	4.85	4.83	4.82	4.80	4.78	4.77	4.75
170	4.73	4.72	4.70	4.68	4.67	4.65	4.63	4.62	4.60	4.58
180	4.57	4.55	4.53	4.52	4.50	4.48	4.47	4.45	4.43	4.42
190	4.40	4.38	4.37	4.35	4.33	4.32	4.30	4.28	4.27	4.25
200	4.23	4.22	4.20	4.18	4.17	4.15	4.13	4.12	4.10	4.08
210	4.07	4.05	4.03	4.02	4.00	3.98	3.97	3.95	3.93	3.91
220	3.90	3.88	3.86	3.85	3.83	3.81	3.80	3.78	3.76	3.75

TEMP. 29.5 C

Absolute
Millivolt

	0	1	2	3	4	5	6	7	8	9
30	7.06	7.04	7.03	7.01	6.99	6.98	6.96	6.94	6.93	6.91
40	6.89	6.88	6.86	6.84	6.83	6.81	6.79	6.78	6.76	6.74
50	6.73	6.71	6.69	6.68	6.66	6.64	6.63	6.61	6.59	6.58
60	6.56	6.54	6.53	6.51	6.49	6.48	6.46	6.44	6.43	6.41
70	6.39	6.38	6.36	6.34	6.33	6.31	6.29	6.28	6.26	6.24
80	6.23	6.21	6.19	6.18	6.16	6.14	6.13	6.11	6.09	6.08
90	6.06	6.04	6.03	6.01	5.99	5.98	5.96	5.94	5.93	5.91
100	5.89	5.88	5.86	5.84	5.83	5.81	5.79	5.78	5.76	5.74
110	5.73	5.71	5.69	5.68	5.66	5.64	5.63	5.61	5.59	5.58
120	5.56	5.54	5.53	5.51	5.49	5.48	5.46	5.44	5.43	5.41
130	5.39	5.38	5.36	5.34	5.33	5.31	5.29	5.28	5.26	5.24
140	5.23	5.21	5.19	5.18	5.16	5.14	5.13	5.11	5.09	5.08
150	5.06	5.04	5.03	5.01	4.99	4.98	4.96	4.94	4.93	4.91
160	4.89	4.88	4.86	4.84	4.83	4.81	4.79	4.78	4.76	4.74
170	4.73	4.71	4.69	4.68	4.66	4.64	4.63	4.61	4.59	4.58
180	4.56	4.54	4.53	4.51	4.49	4.48	4.46	4.44	4.43	4.41
190	4.39	4.38	4.36	4.34	4.33	4.31	4.29	4.28	4.26	4.24
200	4.23	4.21	4.19	4.18	4.16	4.14	4.13	4.11	4.09	4.08
210	4.06	4.04	4.03	4.01	3.99	3.98	3.96	3.94	3.93	3.91
220	3.89	3.88	3.86	3.84	3.83	3.81	3.79	3.78	3.76	3.74

Tables converting millivolt to PH for measurements made with
quinhydrone, Gold electrode and the Calomel saturated half cell.

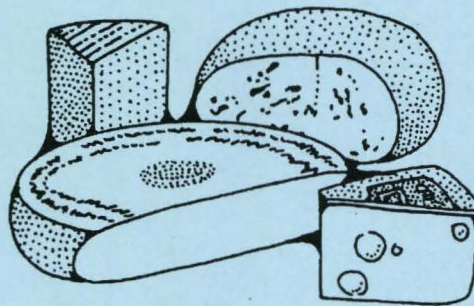
TEMP. 30.0 C

Absolute Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.05	7.03	7.01	7.00	6.98	6.96	6.95	6.93	6.91	6.90
40	6.88	6.86	6.85	6.83	6.81	6.80	6.78	6.76	6.75	6.73
50	6.71	6.70	6.68	6.66	6.65	6.63	6.61	6.60	6.58	6.56
60	6.55	6.53	6.52	6.50	6.48	6.47	6.45	6.43	6.42	6.40
70	6.38	6.37	6.35	6.33	6.32	6.30	6.28	6.27	6.25	6.23
80	6.22	6.20	6.18	6.17	6.15	6.13	6.12	6.10	6.08	6.07
90	6.05	6.03	6.02	6.00	5.98	5.97	5.95	5.93	5.92	5.90
100	5.88	5.87	5.85	5.83	5.82	5.80	5.78	5.77	5.75	5.73
110	5.72	5.70	5.68	5.67	5.65	5.63	5.62	5.60	5.58	5.57
120	5.55	5.53	5.52	5.50	5.48	5.47	5.45	5.43	5.42	5.40
130	5.38	5.37	5.35	5.33	5.32	5.30	5.28	5.27	5.25	5.23
140	5.22	5.20	5.18	5.17	5.15	5.13	5.12	5.10	5.08	5.07
150	5.05	5.03	5.02	5.00	4.98	4.97	4.95	4.93	4.92	4.90
160	4.88	4.87	4.85	4.83	4.82	4.80	4.78	4.77	4.75	4.73
170	4.72	4.70	4.68	4.67	4.65	4.63	4.62	4.60	4.58	4.57
180	4.55	4.53	4.52	4.50	4.48	4.47	4.45	4.43	4.42	4.40
190	4.38	4.37	4.35	4.34	4.32	4.30	4.29	4.27	4.25	4.24
200	4.22	4.20	4.19	4.17	4.15	4.14	4.12	4.10	4.09	4.07
210	4.05	4.04	4.02	4.00	3.99	3.97	3.95	3.94	3.92	3.90
220	3.89	3.87	3.85	3.84	3.82	3.80	3.79	3.77	3.75	3.74

TEMP. 30.5 C

Absolute Millivolt	0	1	2	3	4	5	6	7	8	9
30	7.04	7.02	7.00	6.99	6.97	6.95	6.94	6.92	6.90	6.89
40	6.87	6.85	6.84	6.82	6.80	6.79	6.77	6.75	6.74	6.72
50	6.70	6.69	6.67	6.65	6.64	6.62	6.60	6.59	6.57	6.55
60	6.54	6.52	6.50	6.49	6.47	6.45	6.44	6.42	6.40	6.39
70	6.37	6.35	6.34	6.32	6.31	6.29	6.27	6.26	6.24	6.22
80	6.21	6.19	6.17	6.16	6.14	6.12	6.11	6.09	6.07	6.06
90	6.04	6.02	6.01	5.99	5.97	5.96	5.94	5.92	5.91	5.89
100	5.87	5.86	5.84	5.82	5.81	5.79	5.77	5.76	5.74	5.72
110	5.71	5.69	5.67	5.66	5.64	5.62	5.61	5.59	5.57	5.56
120	5.54	5.52	5.51	5.49	5.47	5.46	5.44	5.42	5.41	5.39
130	5.37	5.36	5.34	5.32	5.31	5.29	5.28	5.26	5.24	5.23
140	5.21	5.19	5.18	5.16	5.14	5.13	5.11	5.09	5.08	5.06
150	5.04	5.03	5.01	4.99	4.98	4.96	4.94	4.93	4.91	4.89
160	4.88	4.86	4.84	4.83	4.81	4.79	4.78	4.76	4.74	4.73
170	4.71	4.69	4.68	4.66	4.64	4.63	4.61	4.59	4.58	4.56
180	4.54	4.53	4.51	4.49	4.48	4.46	4.44	4.43	4.41	4.39
190	4.38	4.36	4.34	4.33	4.31	4.29	4.28	4.26	4.25	4.23
200	4.21	4.20	4.18	4.16	4.15	4.13	4.11	4.10	4.08	4.06
210	4.05	4.03	4.01	4.00	3.98	3.96	3.95	3.93	3.91	3.90
220	3.88	3.86	3.85	3.83	3.81	3.80	3.78	3.76	3.75	3.73

**ANALYZING MANUFACTURING
DATA TO IMPROVE THE
YIELD OF A CHEESE PLANT**



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ANALYZING MANUFACTURING DATA TO IMPROVE THE YIELD OF A CHEESE PLANT¹

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Foss Food Technology Corporation

Abstract.

Some form of batch standardization or the increasing use of on-line standardization systems is now an established practice in the majority of cheese plants. The constantly varying parameters involved in the productivity and profitability of the plant make it essential to be able to estimate which mode of standardization, if any, should be used at any particular time. The purpose of standardization is to modify the casein/fat ratio (C/F) of the milk to satisfy specific requirements for the type of cheese being manufactured. Since the end result can be achieved by different means, it is necessary to investigate the consequences of selecting a particular mode of standardizing from both a productivity and financial point of view. A technique has been developed that relates the variation in C/F to yield as well as profit margin, and a simplified approach to estimating the impact of these changes will be described. The purpose is to provide a tool that will enable the cheesemaker to decide which method of standardizing the cheesemilk will enhance plant performance. The cheesemaking process is complex and is further complicated by the seasonal variations in the composition of the raw materials, and by other variables that affect the productivity of the plant. A mathematical model, titled "Snapshot Normalization", that addresses this problem will be described. The purpose is to illustrate and quantify the significance of each of the different mechanisms within the model that are causing the yield to vary. This will provide a means of measuring plant performance.

1.0 Estimating Cheese Yield Using a linear Equation.

In order to measure the efficiency of the cheesemaking process, it is necessary to relate the weight of milk in the vat to the weight of cheese produced. This measure of efficiency is commonly referred to as percentage yield or productivity, and is calculated as the kg of cheese per 100 kg of milk. An empirical formulae was developed by Van Slyke (1), which provided a means of calculating cheese yield for cheddar cheese. This formulae shown in equation 1 will be used as a basis for discussion.

¹ Presented by David McKenna, Technical Director, Foss Food Technology Corporation, at the Twelfth Biennial Cheese Industry Conference, Utah State University, Logan, Utah, August 20, 1996.

$$Y = (0.93F + C - 0.1) 1.09/(1-M) \dots\dots\dots [1]$$

where F = % Fat in the cheesemilk.
 C = % Casein in the cheesemilk.
 M = kg of water per kg of cheese.

It was explained by McKenna (2) how a measure of the rate of change of yield as a function of the fat, casein, and moisture can be obtained from the first partial derivative, and these terms can be combined to generate a linear form of the yield equation:

$$Y = X1.F + X2.P + X3.M -K \dots\dots\dots [2]$$

and a typical equation for cheddar cheese could be written as follows:

$$Y = 1.64F + 1.76C + 0.16M - 6.25 \dots\dots\dots [3]$$

The influence of moisture and fat retention on the yield equation coefficients can be demonstrated by examining the Van Slyke type equation developed by Brown (3) for mozzarella cheese, see equation 4:

$$Y = (0.88F + C - 0.02)*1.12 / (1-M) \dots\dots\dots [4]$$

Equation 5 is the equivalent linear equation, and it can be seen that compared to the linear equation for cheddar cheese, the coefficients have all increased:

$$Y = 1.86F + 2.11C + 0.20M - 9.26 \dots\dots\dots [5]$$

This means that as the moisture in the cheese increases, small variations in all the independent variables in the equation have a greater impact on the yield, and control of these parameters becomes increasingly important.

2.0 The Impact of Modifying the Component Concentrations on Productivity and Profit Margin.

The effect of changing the component concentration will now be examined. Fig. 1 shows the relationship between Yield and the C/F ratio as the latter is changed by adding cream and/or condensed skim to the raw milk, or separating cream from the raw milk. The origin of the graph coincides with the C/F ratio of the raw milk (0.66) before it is standardized.

As the value of C/F is modified, the yield is plotted for the different methods of standardization. Adding or separating a single commodity is termed Single Standardization. It can be seen that the yield increases as the C/F is reduced to 0.61 by cream fortification, or as the C/F is raised to 0.71 by the addition of condensed skim. Also, the yield decreases as the C/F is increased to 0.71 by cream separation.

This graph also shows the result of Double Standardization. This term describes the method of standardizing where both the fat and casein levels are adjusted. In this example we are using cream with a fat concentration of 38% and casein concentration of 1.5%, and condensed skim with a casein concentration of 8.1% and a fat concentration of 0.15%. It can be seen that adding condensed skim does not significantly increase the yield as the C/F ratio is adjusted from 0.66 to 0.71, and this is because the increase in casein concentration is offset by a reduction in fat concentration (fat dilution). When the C/F ratio reaches 0.71, it can be seen that the effect of adding the cream now takes over and causes a turning point in the C/F ratio. The yield now increases at a faster rate as the C/F ratio is reduced to 0.61. The main advantage of adding condensed skim is that it acts as a substrate for the addition of more cream. So effectively we have increased the total solids of the cheesemilk, and hence the productivity of the vat. Fig. 1 demonstrates this point and shows how Double Standardization can produce the same C/F ratio in the cheesemilk, and potentially the same FDB in the finished product. Some cheese plants in the United States are now using this technique to raise the total solids of the cheesemilk to between 14 and 15%.

Fig. 2 illustrates the variation of **\$ Value Ratio** as a function of the same change in C/F. The term **\$ Value Ratio**, which relates yield and material pricing, is defined below in equation 6. It can be seen that if the value of the cheese increases faster than the cost of the raw materials used in the process, this value will increase, and obviously decrease if the situation is reversed.

$$\text{\$ Value Ratio} = (\text{Value of the cheese})/(\text{Cost of Raw Materials}) \dots\dots\dots [6]$$

It can be seen in Fig. 2, that for this yield/pricing relationship, as the C/F ratio is adjusted by separating cream or adding condensed skim, the **\$ Value ratio** is decreased, whereas it is increased by the addition of cream. For this example it means that the profit margin is increased by adding cream, and reduced by separation or adding condensed skim. In the case of separation the value of the cheese has decreased (due to a decrease in yield), at a faster rate than the decrease in the cost of the raw materials, which has also been adjusted to take into account the value of the sale of the surplus cream. As we have seen the addition of the condensed skim will slightly increase the yield, and hence the value of the

cheese produced. In this example the cost of the raw materials has increased by a greater amount, so the value of the **\$ Value Ratio** or percentage profit margin is decreased. However, this changes to an increase in margin by the addition of the cream in the second leg of the Double Standardization.

As the cost of the condensed skim increases the slope of the condensed skim line will increase negatively until it coincides with the line that represents separation. At this point Double Standardization will produce the same **\$ Value Ratio** as the addition of cream, but with a higher yield. If the cost of the condensed skim continues to increase the **\$ Value ratio** will now be less than that for the addition of cream only, in spite of the higher yield. This mechanism is illustrated in Fig. 3. It should also be noted that the **\$ Value Ratio** is also directly proportional to changes in the selling price of the cheese, so this is indeed a complicated relationship.

The arguments addressed in the preceding paragraphs were meant to show the complex nature of some of the factors that can significantly impact the business of cheesemaking. This suggests that decision making process should be in place to enable the cheesemaker to quickly react to a particular set of circumstances, and if necessary, make changes to the method of standardizing the cheesemilk. This must include the capability to perform standardizing calculations that will estimate the best approach in any current situation. This requirement has been previously addressed (2) but in general was thought too complicated. A simplified means of performing these calculations within an Excel spreadsheet in the Windows environment has now been developed. The main advantage of this new approach is that the user is not restricted within the confines of a packaged program. This means that the basic application can easily be further developed to fit the unique requirements of a particular process on an individual basis. Some worked examples are illustrated in Figs. 4, 4a, 4b, and 4c, and these will now be considered.

Fig. 4 shows the screen titled "Raw Milk Potential", and estimates the performance if the milk in the vat has not been standardized. It includes the raw milk component concentrations, the material prices, vat size, and target moisture value that will be used in all four examples. All these values can be changed to fit different circumstances. In this example the C/F ratio of the raw milk was calculated to be 0.66 with an equivalent FDB of 52.1%. Using the linear equation described in the next section, the yield was estimated to be 10.03, with an estimated **Profit per vat** of \$360.22 and **\$ Value Ratio** of 1.06.

This section of the program allows the user to input both the required C/F ratios for the different stages of the standardizing process. The component concentrations of fat and

casein in the commodities being used to standardize the cheesemilk should also be entered on this screen. Referring to Fig. 1, the **HI** title refers to the adjustment of the **C/F** ratio to the high point by the addition of condensed skim, while the **LO** refers to the adjustment to the low point by the addition of cream. Values of **HI** and **LO** can be selected to estimate the potential of all the different modes of standardizing using these commodities. For the addition of cream only, the **HI** value retains the default raw milk value of the **C/F**.

An example of this method of standardizing is shown in Fig. 4a, and it can be seen that a value of 0.61 has been selected for **LO**. This would increase the Total Solids in the milk to 12.58 and raise the FDB in the finished product to 54.12. The new fat and protein concentrations are shown and these could be used as target values for the on-line standardizing system. Alternatively, for batch standardizing, the pounds of cream that need to be added to the vat while maintaining the same vat size is also listed, plus the increased cost of the raw materials. The yield is now estimated at 10.50 and the **\$ Value Ratio** has increased from the raw milk value of 1.056 to 1.091. **Delta Yield** and **Delta Profit** represent the change in yield and profit per vat as compared to the raw milk performance.

The potential of Double Standardizing using the **HI** and **LO** targets shown in Fig. 2 as 0.71 and 0.61 respectively, is illustrated in Fig. 4b. The Total Solids in the cheesemilk has increased to 12.97 with fat and casein concentrations of 4.12 and 2.51 respectively. The required weights and total cost of adding both commodities is listed and this has raised the yield to 11.01. The **\$ Value Ratio**, **Delta Yield**, and **Delta Profit** all show significant increases, so in this case if the commodities were available, Double Standardization would be the obvious choice. Fig. 4c considers the same standardizing objectives but the cost of the condensed skim has been raised from \$0.30 to \$0.35 per pound. It can be seen that the **\$ Value Ratio** and the **Delta Profit** are both significantly affected, as was illustrated in Fig. 3. These worked examples demonstrate the need for continuously monitoring the efficiency of the process and also the continuing impact of the continuously changing market conditions.

3.0 Theoretical Considerations of Snapshot Normalization.

As shown in Fig. 5, the yield of a cheese plant can vary quite considerably over the period of a year. A mathematical model, titled "Snapshot Normalization", has been developed to help illustrate and quantify the significance of each of the different mechanisms that are causing the yield to vary. This treatment uses monthly average values to try and eliminate the short term effects due to errors that may have occurred in sample analysis and

estimates of plant performance. Once the causes and financial consequences have been clearly identified, this will enable the relevant corrective action to be taken. Initially, the independent variables that will be examined in the model will be limited to the following:

1. The seasonal variations of the fat and casein in the raw milk.
2. The retention of fat in the curd.
3. The concentration of fat and casein in the cheesemilk.
4. The moisture in the finished product.

3.1 Seasonal Variations.

The theoretical reasoning for the development of a linear equation to estimate cheese yield was reported in (2). The general form of this equation, which is shown in equation 2, allows the change in yield due to a change of fat or casein in the cheesemilk, or a change in moisture in the finished product to be easily estimated. For example, the change in yield dY_f , due to a small change in fat dF , can be expressed as:

$$dY_f = X_1 \cdot dF \quad \dots\dots\dots [7]$$

Similarly, for a small change in the casein concentration:

$$dY_c = X_2 \cdot dC \quad \dots\dots\dots [8]$$

and for a small change in the moisture content:

$$dY_m = X_3 \cdot dM \quad \dots\dots\dots [9]$$

For many types of cheese, such as mozzarella or the low fat varieties, where the casein/fat ratio Z has to be modified, the relationship for a specific recipe can be expressed as:

$$Z = C/F \quad \dots\dots\dots [10]$$

In a well controlled process this value of Z will be constant for a specific type of cheese, but will differ for the different cheese types. It has been reported by Barbano (4) that both the fat and casein concentrations in the raw milk are subject to seasonal variations, so maintaining a constant value of Z becomes a seasonal challenge. For specialty types of cheese the value of Z for the cheesemilk is normally greater than that for the raw milk, and

so the ratio can be adjusted by reducing the fat concentration. Alternatively, for cheddar type cheeses, the cheesemaker may wish to lower this ratio in order to maximize the **FDB** in the finished product; and hence has to fortify the cheesemilk with added cream. In either case the amount of cream, separated or added, will be governed by the casein concentration of the raw milk. This limitation can be overcome by simultaneously increasing the casein level, which has been referred to as Double Standardizing. So, it can be argued that the controlling parameter of the standardization process is the casein concentration in the raw milk. By differentiating equation 10, it can be shown that for a seasonal change in the casein concentration **dC**, the required change in the fat concentration to maintain the same value of **Z** can be written as follows:

$$dF = dC/Z \quad \text{..... [11]}$$

By combining equations 7 and 8, it can be shown that the change in yield resulting from the seasonal concentration changes in both these components can be written as:

$$dYs = X1.dF + X2.dC \quad \text{..... [12]}$$

Substituting for **dF** from equation 11 the change in yield can now be expressed as a function of the controlling parameter casein:

$$dYs = dC.(X1 /Z + X2) \quad \text{..... [13]}$$

If we now consider the seasonal changes in casein shown in Fig. 6 in monthly increments, we can write:

$$dC = (C \text{ monthly avg.} - C_{max}) \quad \text{..... [14]}$$

where **C_{max}** = The highest monthly average casein value.

and **C monthly avg.** = The average value for any other month.

By substituting this value of **dC** in equation 13, the seasonal variation in yield **dYs** for a specific month can be written as:

$$dYs = (C \text{ monthly avg.} - C_{max}).(X1 /Z + X2) \quad \text{..... [15]}$$

It can be seen that the **dYs** can be reduced to zero by fortifying the monthly casein level to equal **C_{max}**, and that even heavier fortification would result in a positive gain in yield. As

has already been discussed the commodity costs will determine whether this approach is economically feasible.

3.2 Fat Retention.

The second mechanism to be examined is the retention **R** of fat in the cheese. It was described in (2) that the following equation defines the relationship between **C/F** and **FDB**:

$$Z = (100/FDB-1)/k \quad \dots\dots\dots [17]$$

where **k** = a constant for a particular type of cheese.

But this expression does not take into account variations in fat retention, so it was also reported that this is not only a major source of error in yield predictions, but it also reduces the possibility of achieving a consistent **FDB** in the finished product. This is because the relationship between the control point **Z** and the **FDB** has been disrupted by deviations in the fat retention. A value of **R** can be obtained from the following expression:

$$R = Fc/Fm \cdot Y \quad \dots\dots\dots [18]$$

where **Fc** = % Fat in the cheese.
and **Fm** = % Fat in the cheesemilk.
and **Y** = Yield.

Using this formulae the monthly average values of fat retention were calculated and are recorded in Table 1, and plotted in Fig. 7. It can be seen that a high of 95.58 was achieved in month 6. It was decided to use the simple criteria, that if this level of fat retention can be achieved for a whole month, then by careful control, it should be possible to achieve a similar value for the rest of the year. In other words **Rmax** is now the benchmark and the target to achieve. Obviously, as the control over the fat retention improves, the value of **Rmax** would be expected to increase, and hence the fat coefficient value **X1** in the linear equation. The values of retention **R**, for the other 11 months were therefore normalized against **Rmax**, and the resulting loss in yield can be calculated using the following equation:

$$dYr = (R/Rmax - 1) \cdot X1 \cdot Fm \quad \dots\dots\dots [19]$$

where **Fm** = Average fat for the month.
and **X1** = The fat coefficient from the linear equation.

If one were to substitute the accepted maximum achievable value for **Rmax** in the equation, the value of **dYr** could be calculated for the whole 12 months.

3.3 Target Values for Fat, Casein, and Moisture.

Unlike the two variables discussed so far, the cheesemaker will usually be trying to standardize to specific target values for the fat in the cheesemilk, and moisture in the finished product. Also, in the case of Double Standardization he will have a target value of casein in the cheesemilk. If he misses the target on any of these parameters there will be a gain or loss in yield depending on whether he overshoots or undershoots. There is also the added confusion, that if he shoots high on fat for example, the resultant increase in yield could be canceled out by a low moisture value. Since each of these parameters can be considered as an independent variable, the monthly average values were normalized against the respective Target values using the following expressions.

$$dY_f = (F - F_t) \cdot X_1 \quad \dots\dots\dots [20]$$

$$dY_c = (C - C_t) \cdot X_2 \quad \dots\dots\dots [21]$$

$$dY_m = (M - M_t) \cdot X_3 \quad \dots\dots\dots [22]$$

where **F**, **C**, and **M** are the monthly average values.
 and **F_t**, **C_t**, and **M_t** are the target values.
 and **X₁**, **X₂**, and **X₃** are the respective coefficients from the linear equation.

It can be seen that the values of **F**, **C**, and **M** as they relate to their respective target values will determine whether their individual impact on yield is negative or positive.

4.0 Practical Application of the Snapshot Model.

The numbers listed in Table 1 represent the monthly averages collected over a 12 month period, and these will be used to demonstrate the workings of the model. The main purpose is to explain and quantify the reason for the variation in yield shown in Fig. 5, which shows a substantial drop in the summer months, and a gradual recovery as winter approaches.

In order to solve the equations described in the previous section it is required to know the values of the coefficients from the linear equation (**X₁**, **X₂**, and **X₃**). So the first step is to generate a linear equation from the data in Table 1 as follows:

$$Y = 1.6507 * F + 1.7478 * C + 0.1599 * M - 5.7071 \dots [23]$$

where **X1 = 1.6507**
and **X2 = 1.7478**
and **X3 = 0.1599**

The main advantage of normalizing these different parameters with respect to the individual impact on yield, is that their relative significance's can be compared and quantified. The stacked bar graph shown in Fig. 8 illustrates how the impact on yield from these different mechanisms can be easily related. The economic significance of these influences can be checked by converting the loss in yield to the loss in revenue that results from the drop in productivity as shown in Table 2. These two diagrams illustrate the practical aspect of the model, because it has produced a "snapshot" of the process, and enables the following observations.

The major reason for the loss in yield is the seasonal variation of the casein. To prevent this type of loss the cheesemaker would have to consider Double Standardizing the cheesemilk. This immediately raises questions on the economics and logistics of using a concentrated protein source. Obviously, the economical aspects can be investigated using the standardizing section of the software described in section 2.0. It is worth mentioning that if the milk is fortified with casein, **Cmax** will still represent the maximum raw milk value. This means that if the value of **C monthly avg > Cmax**, the bar representing **dYs** would show a positive contribution to yield and this would automatically be quantified in the Season column in Table 2 as an increase in revenue. This point was already explained when the structure of equation 15 was examined.

In order to study the lesser mechanisms more closely, the seasonal effects (**dYs**) have been removed from the stack in Fig 9. It can be seen that in months 2-8 there was a significant loss in yield due to the fat target not being achieved, and for the remaining months the yield benefited by this value being too high. It will also be noted that the variations in fat retention present an opportunity to improve plant performance. Unlike the effect of seasonal changes, both these issues can be classed as process control problems that are directly related to the efficiency of the process, and control of the **FDB**. It should also be noted that for 5 months the losses associated with the low fat levels were further exaggerated by the low levels of retention. Finally it can be seen that the deviations in moisture from the target value were the least significant cause of variability. It should be noted that the numbers for moisture have not been modified to include any bonus that might have been applicable for the higher solids level, but are strictly attributed to the loss or gain of productivity.

Fig. 10 compares the shape of the algebraic sum of the independent variables in the model to the shape of the yield graph from Fig. 6. If the model adequately describes the yield variations, one would expect the shape of the two graphs to be comparable. In this particular case there appears to be a good fit.

Other points to consider include the following:

- a. Although this model has been constructed in terms of monthly averages, to obtain better resolution on the individual mechanisms, it can be operated using weekly increments. This could be an important point in large plants for large volume products because swift action to correct a problem will be required.
- b. The information generated from the model could also be used to try and determine whether the fluctuations are mainly plant specific, product specific, seasonal specific or simply random increments. This could be an important avenue of discovery for companies who operate a number of different plants, or for large volume products in single plants.

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File Ref: Utahst.doc

1. THE PERFORMANCE ANALYZER

MONTH	CHEESE							MONTHLY	VATS	CHEESE
	FAT	CAS	%FAT	MOIST	YIELD	FDB	C/F	RETN	/MONTH	PRICE
1	3.92	241	35.31	34.24	10.49	53.70	0.61	94.49	1120	1.35
2	3.88	242	35.29	34.30	10.39	53.71	0.62	94.50	1116	1.27
3	3.84	241	35.00	34.88	10.42	53.75	0.63	94.97	900	1.26
4	3.72	234	34.93	34.74	9.94	53.52	0.63	93.33	910	1.27
5	3.70	233	34.86	34.56	9.96	53.27	0.63	93.84	884	1.29
6	3.72	237	35.10	34.34	10.13	53.46	0.64	95.58	885	1.33
7	3.87	245	34.89	34.67	10.32	53.41	0.63	93.04	945	1.44
8	3.95	246	35.32	34.74	10.61	54.12	0.62	94.87	957	1.48
9	4.04	247	35.07	34.81	10.87	53.80	0.61	94.36	768	1.46
10	4.01	244	35.11	34.76	10.84	53.82	0.61	94.91	651	1.41
11	4.00	241	35.20	34.69	10.77	53.90	0.60	94.78	839	1.41
12	3.98	242	35.23	34.57	10.69	53.84	0.61	94.63	735	1.40

Table 1

SHORTFALL / WINDFALL

	SHFL \$	SHFL \$	SHFL \$	SHFL \$	Gain/Loss
Month	Fat	Moist.	Reten.	Season	Total
1	40,909	(31,305)	(55,639)	(199,308)	(245,344)
2	(27,223)	(22,701)	(51,417)	(156,576)	(257,916)
3	(44,038)	34,410	(22,821)	(149,894)	(182,343)
4	(51,510)	22,091	(83,121)	(330,126)	(442,665)
5	(54,463)	5,450	(63,255)	(350,848)	(463,116)
6	(99,902)	(15,090)	0	(260,198)	(375,190)
7	(91,821)	18,522	(115,772)	(60,119)	(249,189)
8	(20,706)	27,126	(34,208)	(31,182)	(58,971)
9	52,076	27,853	(47,934)	0	31,995
10	56,481	19,085	(21,326)	(60,753)	(6,513)
11	110,306	17,977	(32,932)	(156,618)	(61,267)
12	65,343	5,770	(33,886)	(113,697)	(76,470)
Total =	(64,548)	109,187	(562,310)	(1,869,318)	(2,386,990)

Table 2

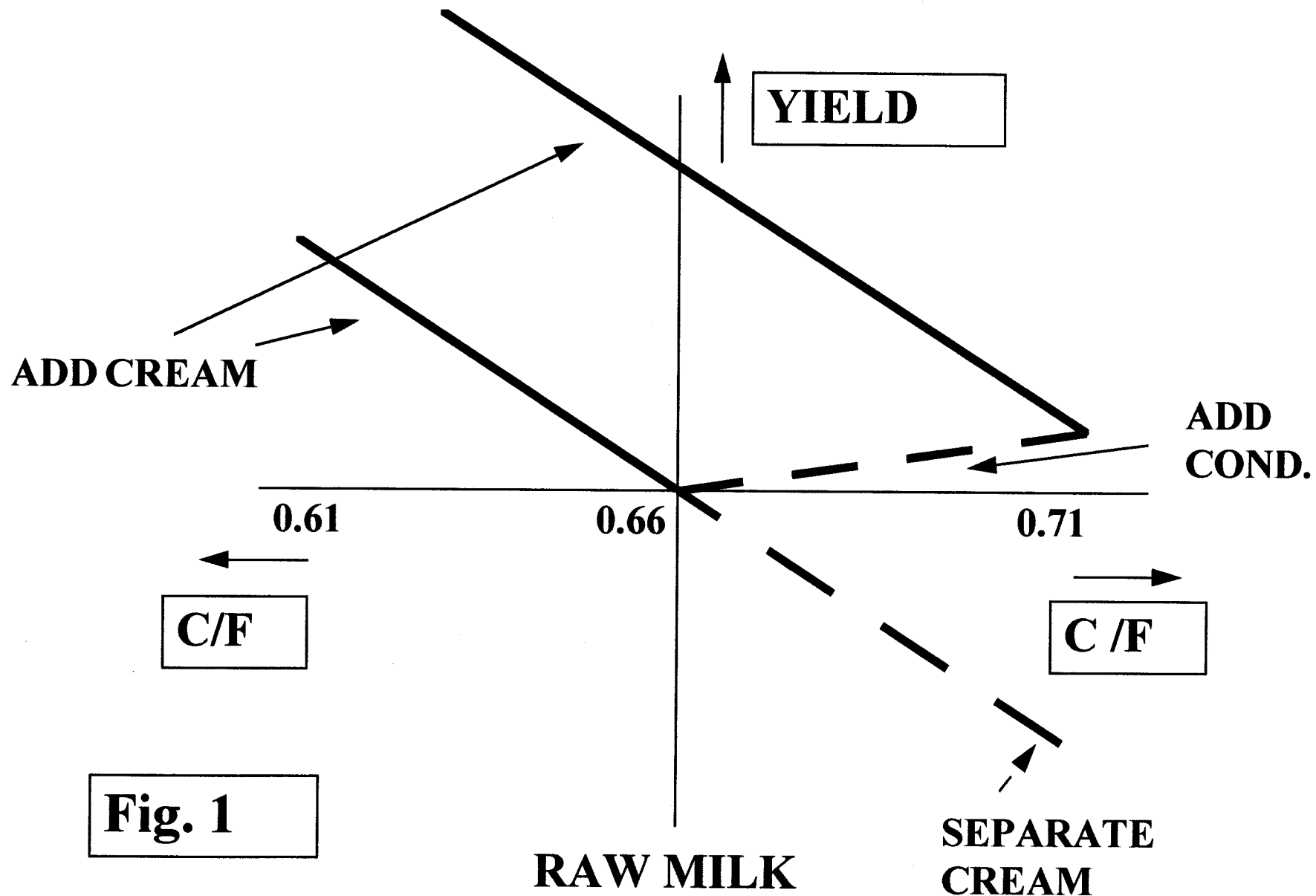


Fig. 1

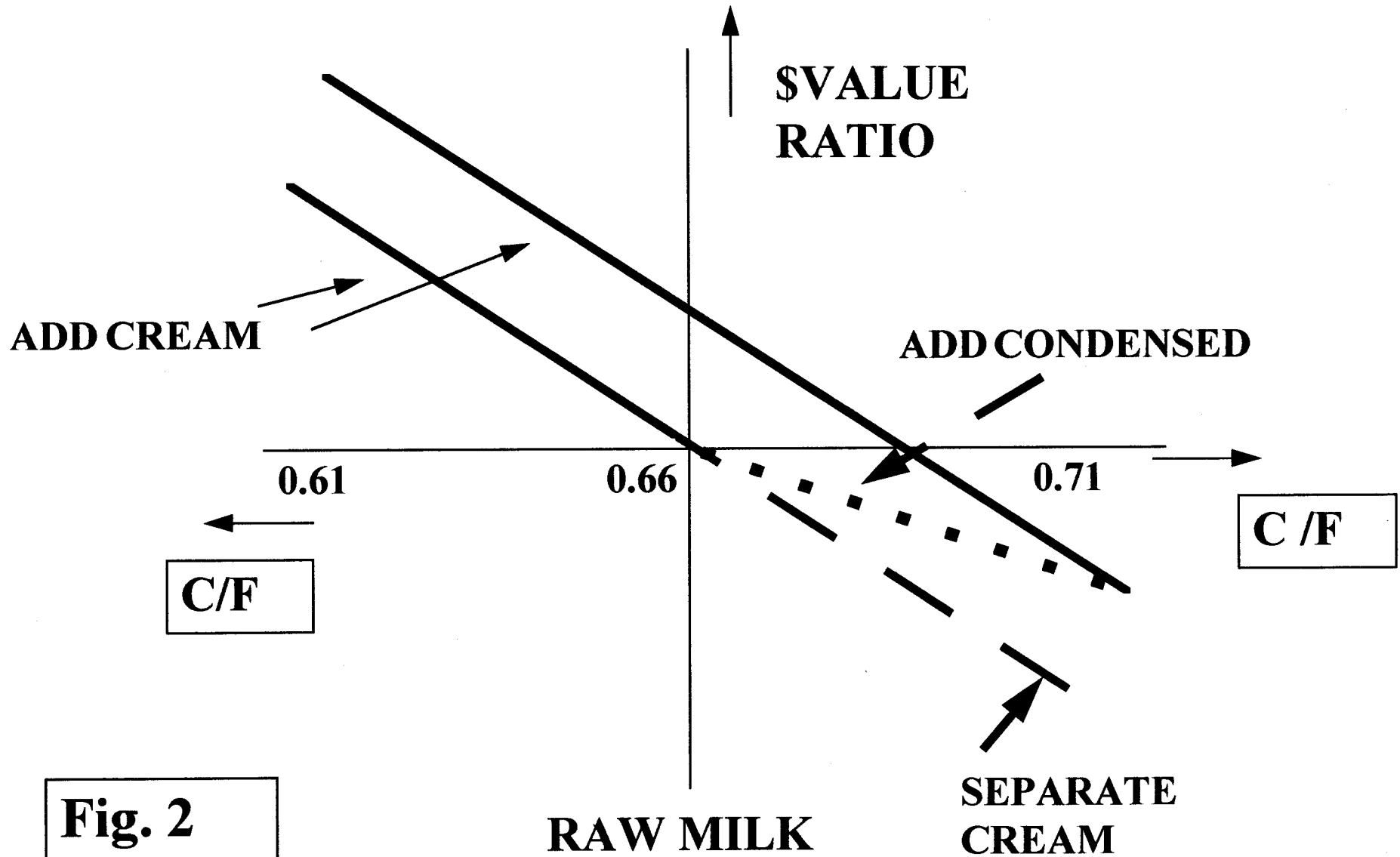


Fig. 2

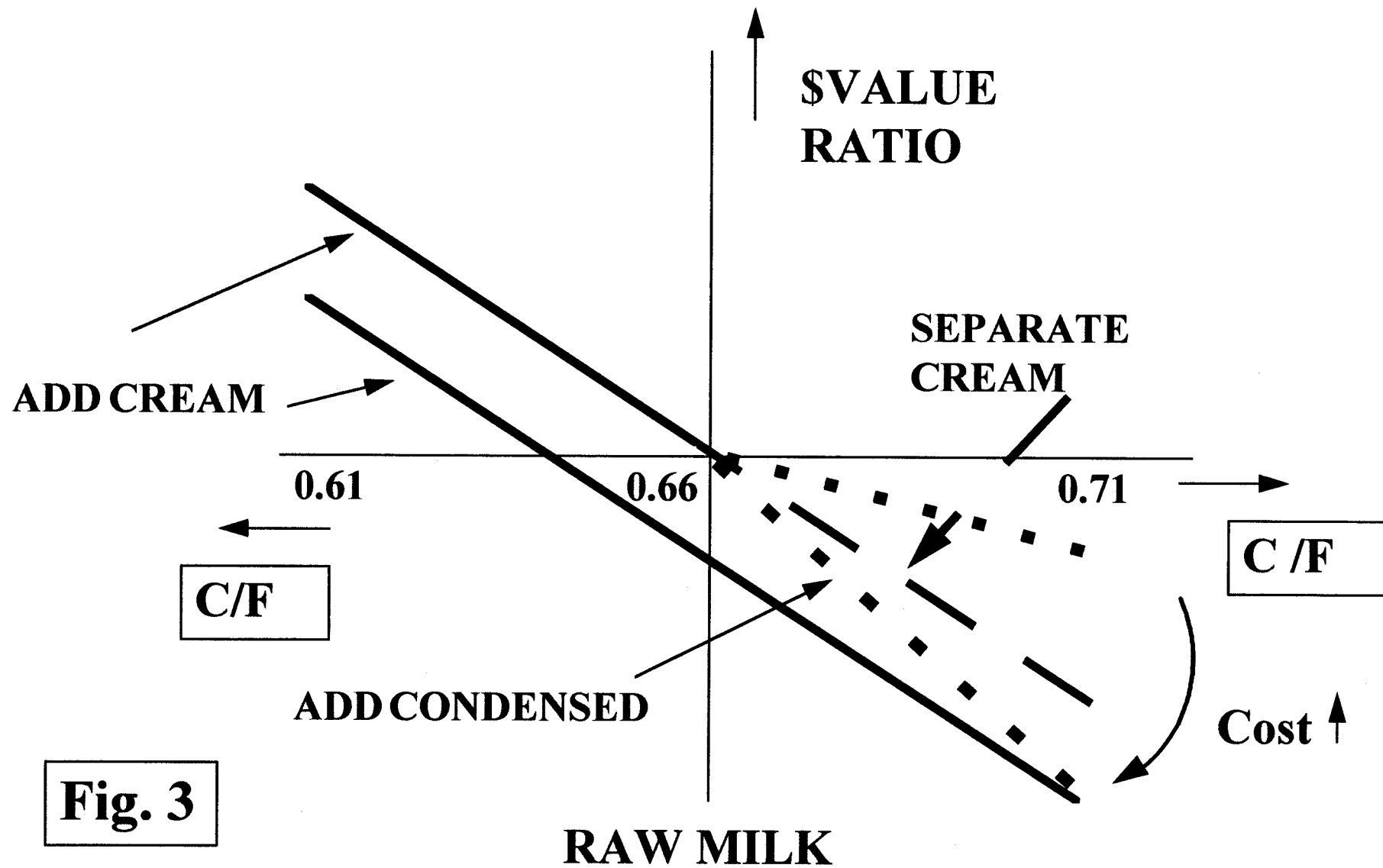


Fig. 3

THE STANDARDIZER

	HI	LO			
ENTER REQUIRED C/F =	0.71	0.61	COMMODITY CONCENTRATIONS		
ESTIMATED T. SOLIDS =		12.97			
ESTIMATED FDB =	54.12			%FAT	%CAS
			CREAM	38	1.5
			COND. SKIM	0.14	8.1

STANDARDIZED FAT	CAS	C/F	LBS CREAM	LBS COND.	COST	EST. YIELD	\$VALUE RATIO
4.12	2.51	0.61	809	1071	355	11.01	1.099

DELTA YIELD	DELTA PROFIT
0.98	304

**Fig. 4b Double Standardization,
(Addition of Cream & Cond. Skim).**

THE STANDARDIZER

	HI	LO			
ENTER REQUIRED C/F =	0.71	0.61	COMMODITY CONCENTRATIONS		
ESTIMATED T. SOLIDS =		12.97			
ESTIMATED FDB =	54.12			%FAT	%CAS
			CREAM	38	1.5
			COND. SKIM	0.14	8.1

STANDARDIZED FAT	CAS	C/F	LBS CREAM	LBS COND.	COST	EST. YIELD	\$VALUE RATIO
4.12	2.51	0.61	809	1071	408	11.01	1.090

DELTA YIELD	DELTA PROFIT
0.98	250

**Fig. 4c Double Standardization,
(Increasing the Cost of Cond. Skim).**

TREND OF YIELD

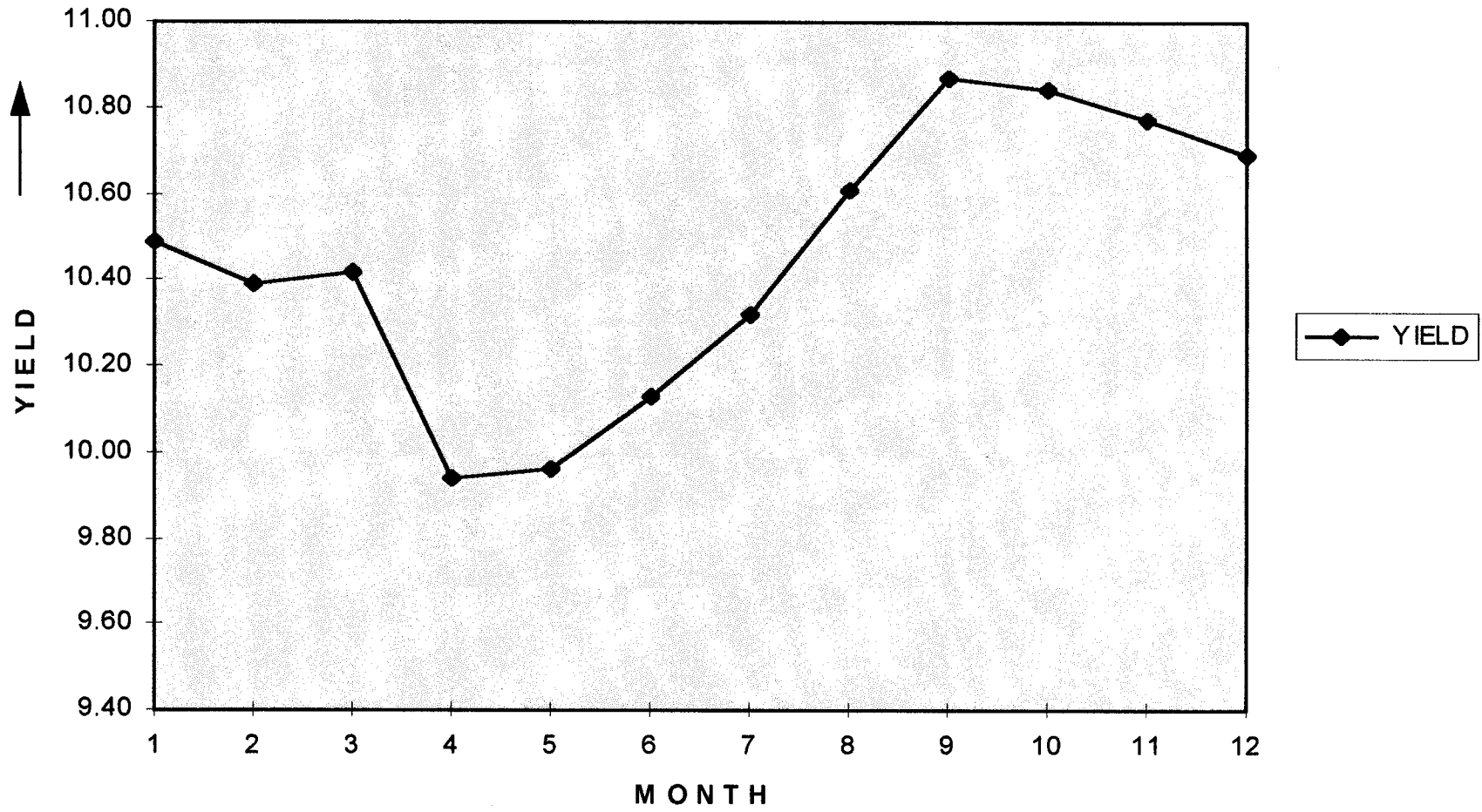


Fig. 5

Monthly Trend of Casein

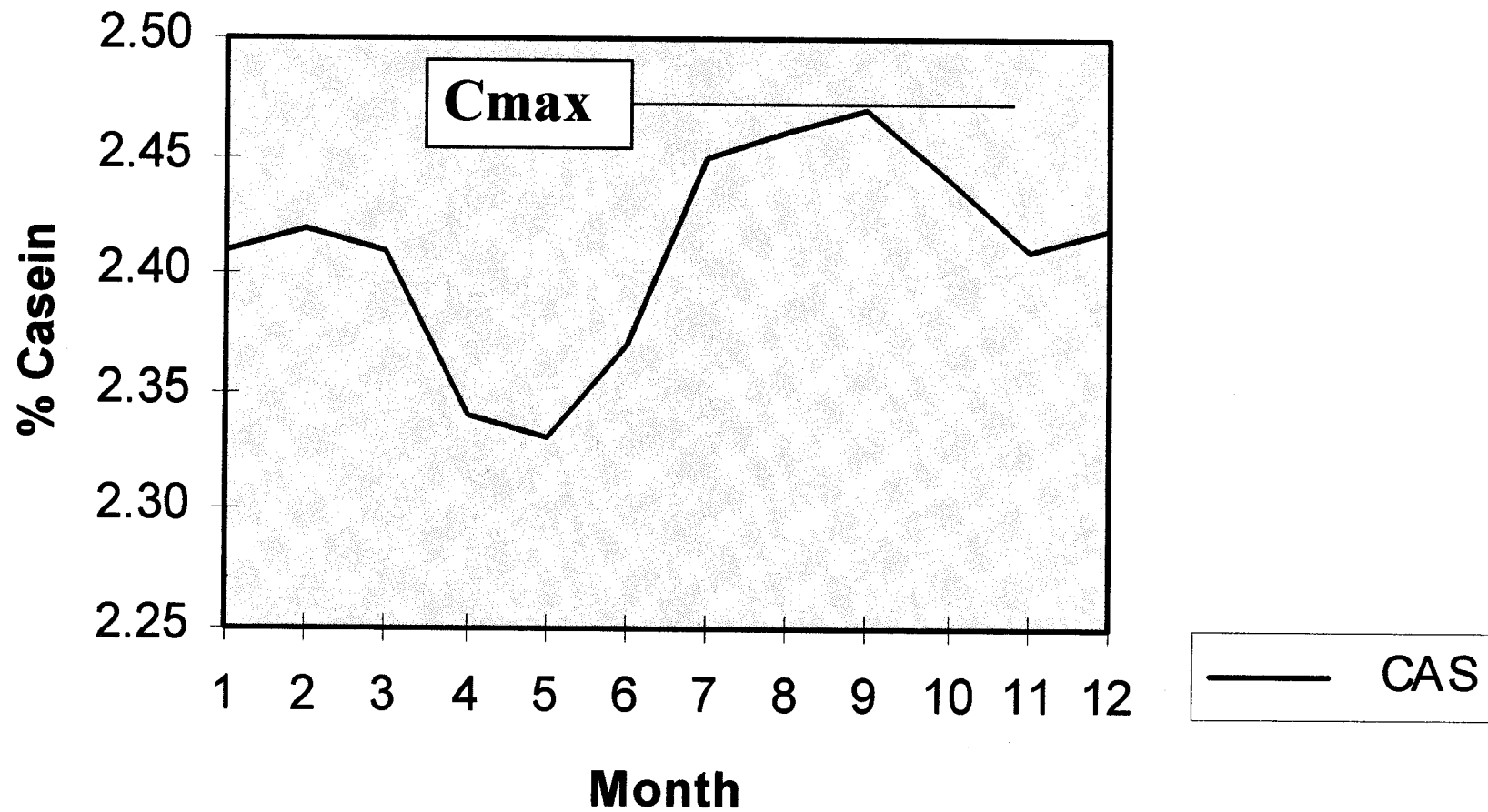


Fig. 6

Monthly Trend of Fat Retention

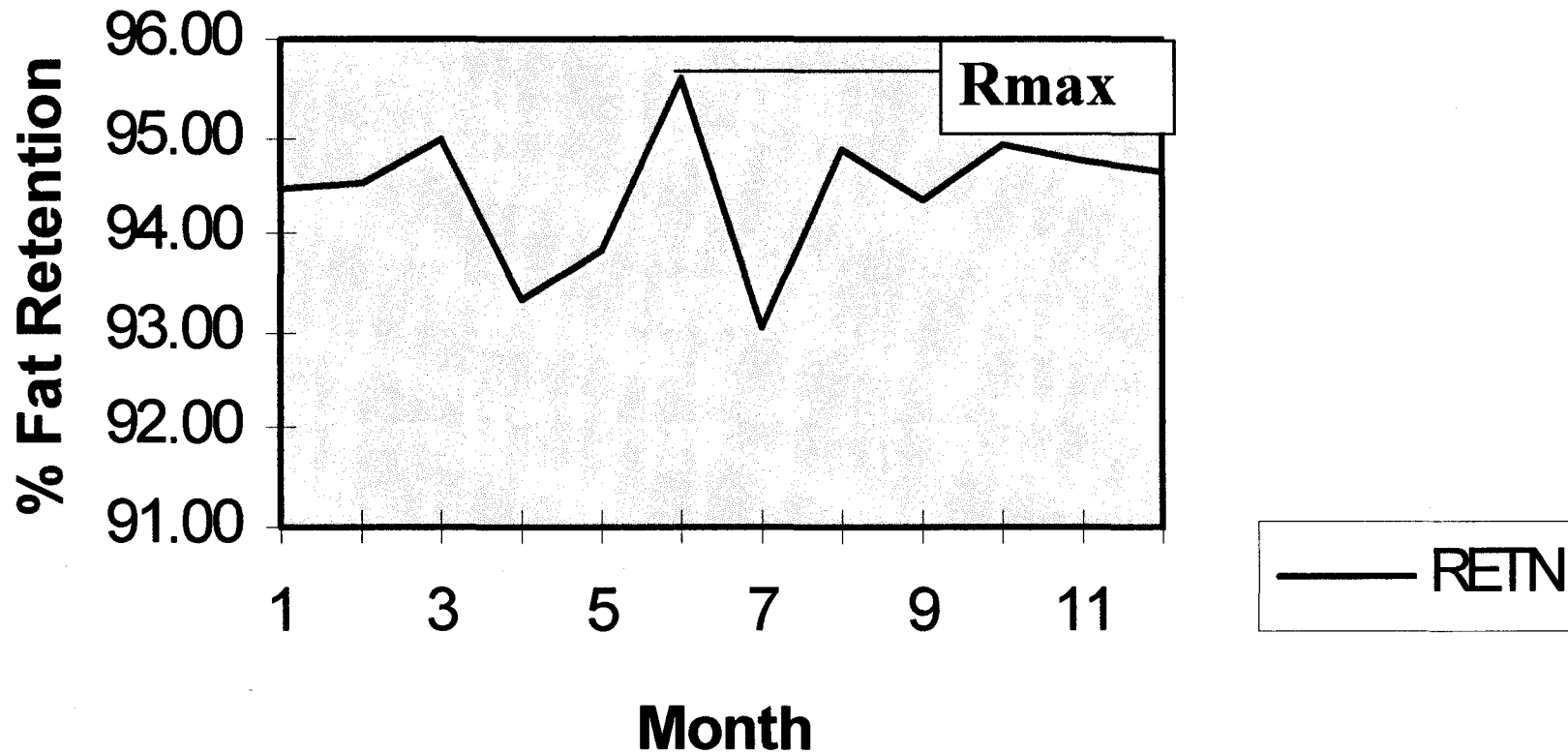
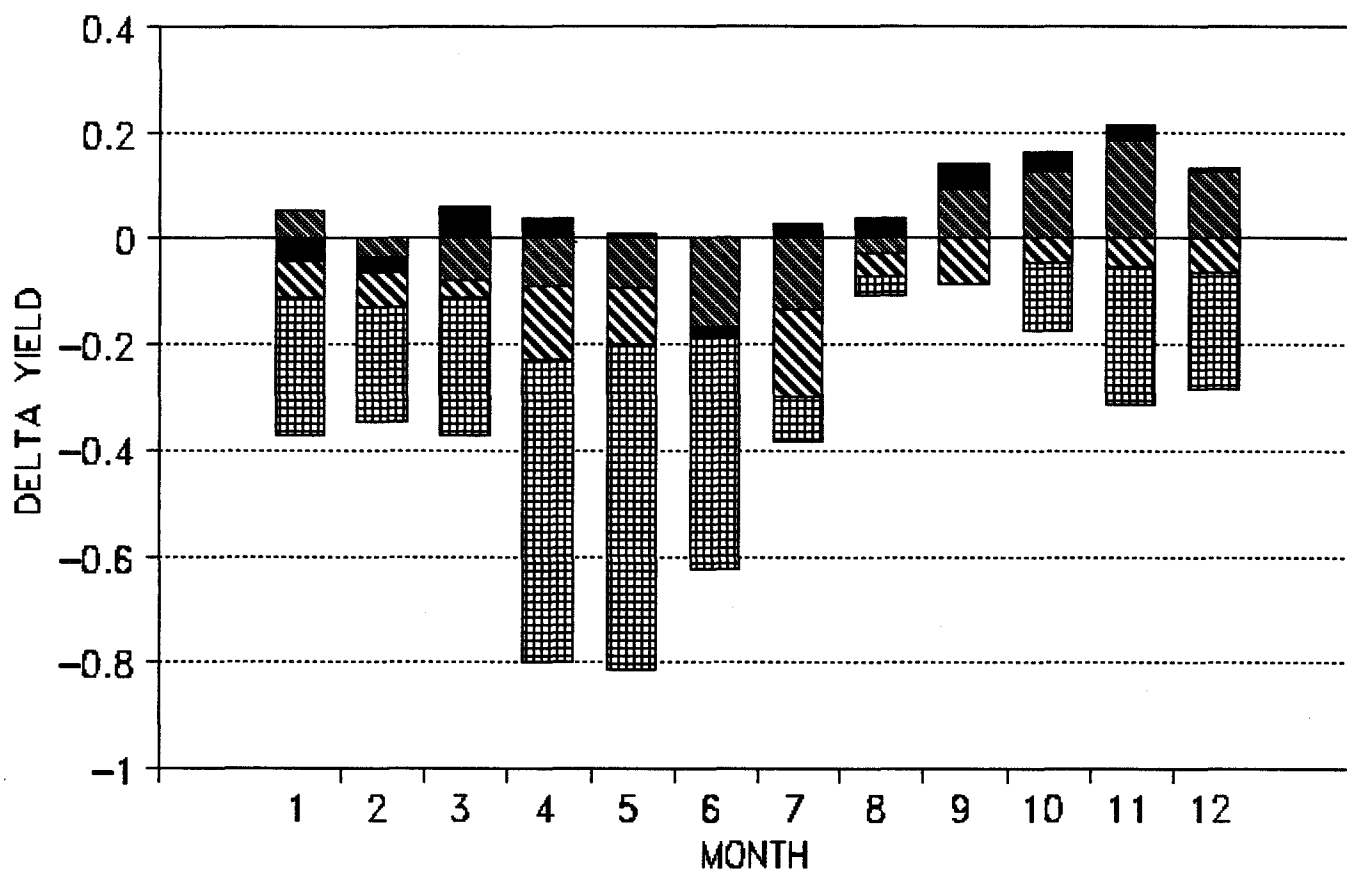


Fig. 7

FIG. 8 SNAPSHOT NORMALIZATION



Raw Milk Potential:

	RAW MILK			RAW MATERIAL PRICES:			Price
MONTH	FAT	PROT	CAS	MILK	CREAM	COND.	CHEESE
1	3.64	3.17	2.41	0.1277	0.3381	0.3	1.345

VAT SIZE = 50000

TARGET MOIST. = 34.5

		EST.	EST.	PROFIT	\$VALUE
MONTH	C/F	FDB	YIELD	/VAT	RATIO
1	0.66	52.08	10.03	360	1.056

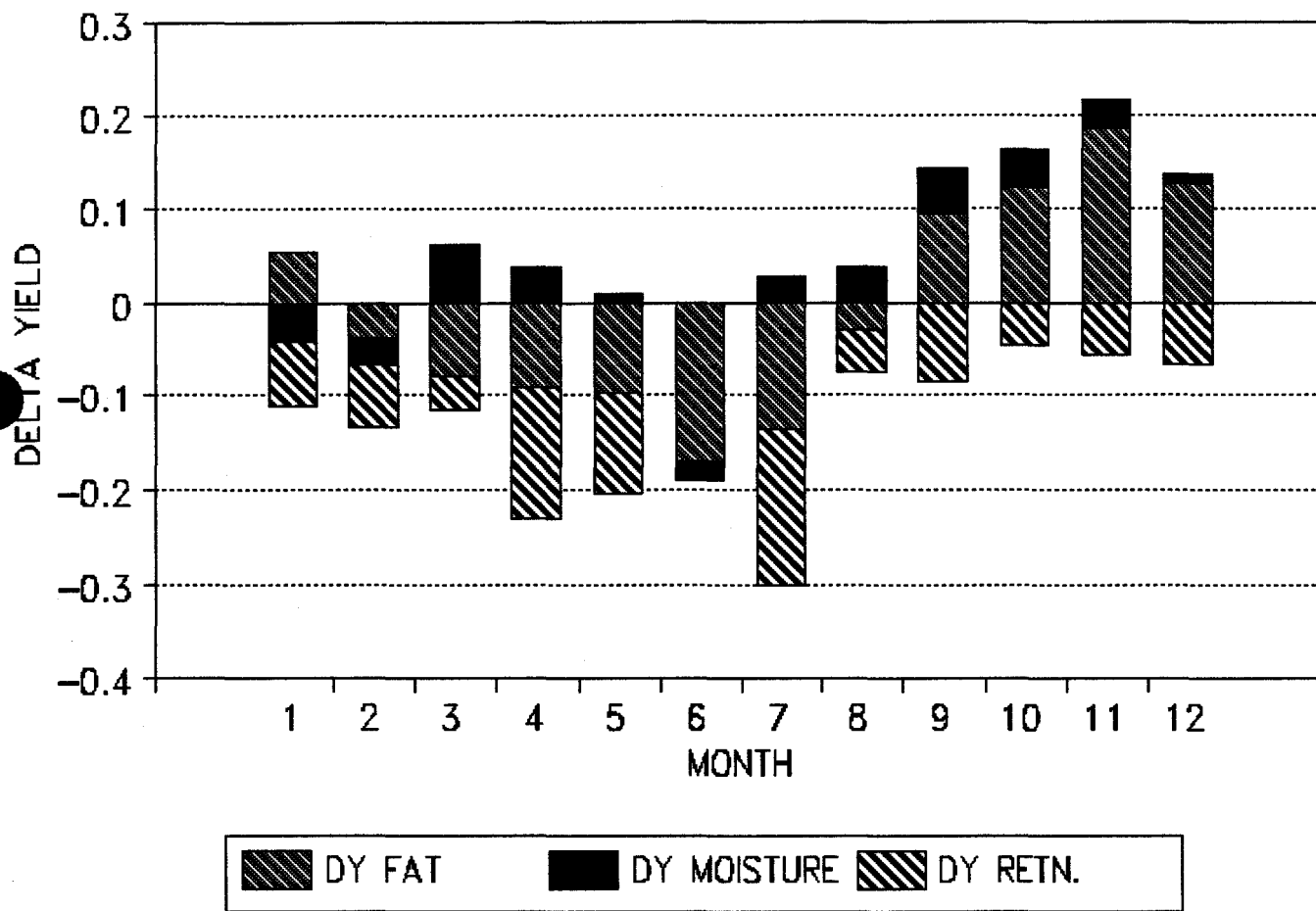
Fig. 4

THE STANDARDIZER

	HI	LO					
ENTER REQUIRED C/F =	0.66	0.61	<u>COMMODITY CONCENTRATIONS</u>				
ESTIMATED T. SOLIDS =		12.58					
ESTIMATED FDB =	54.12			%FAT	%CAS		
			CREAM	38	1.5		
			COND. SKIM	0.14	8.1		
STANDARDIZED FAT	CAS	C/F	LBS CREAM	LBS COND.	COST	EST. YIELD	\$VALUE RATIO
3.94	2.40	0.61	432	0	91	10.50	1.091
					DELTA YIELD	DELTA PROFIT	
					0.48	229	

**Fig. 4a Single Standardization,
(Addition of Cream).**

FIG. 9 SNAPSHOT NORMALIZATION
(WITHOUT FORTIFICATION EFFECT)



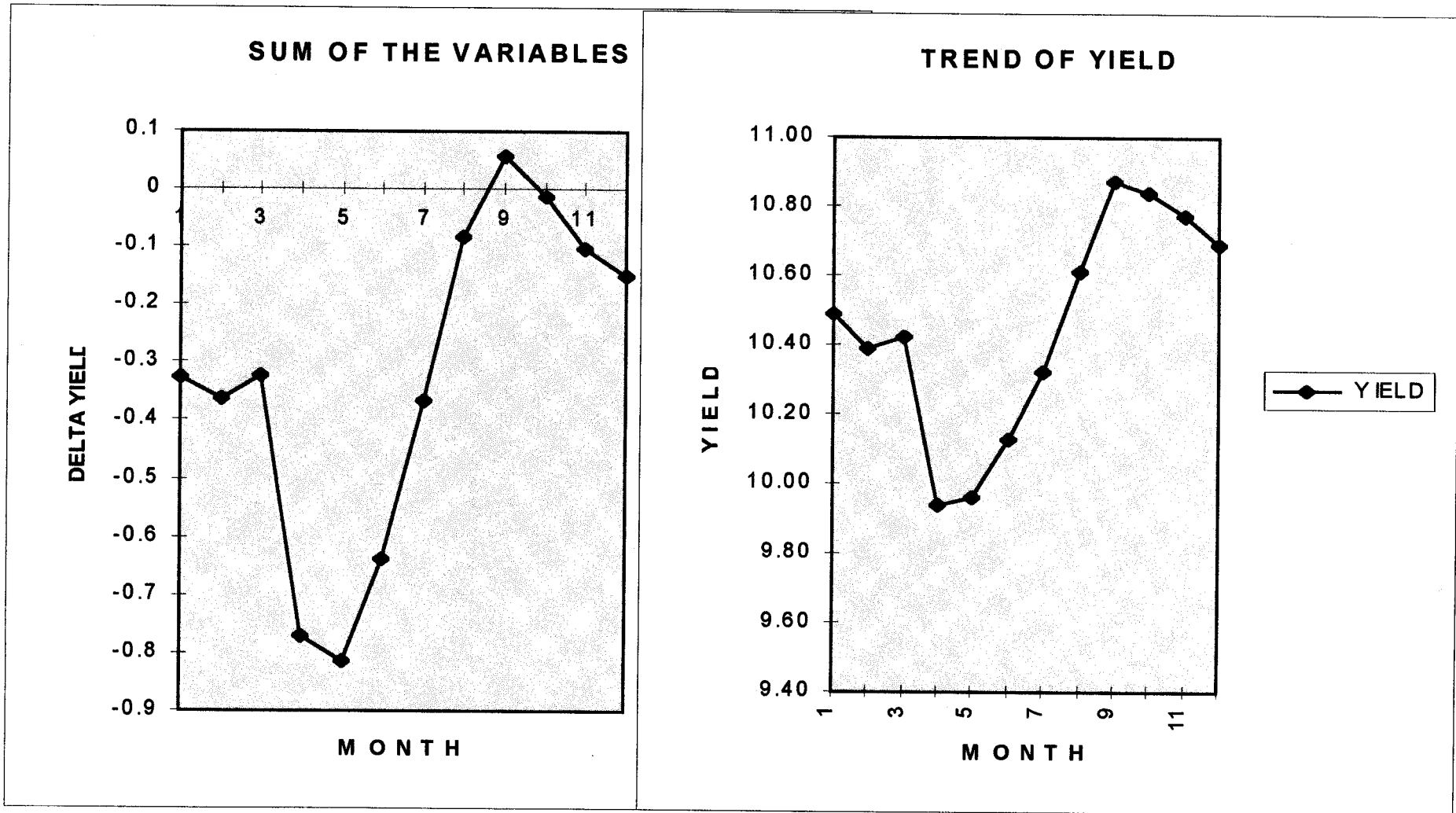
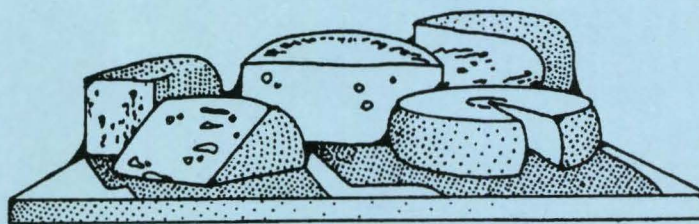


Fig. 10

USING STATISTICAL PROCESSES TO CONTROL PRODUCT QUALITY



STEVE LARSEN
HILMAR CHEESE

USING STATISTICAL PROCESSES TO CONTROL PRODUCT QUALITY

Steve Larsen
Hilmar Cheese

Utah State University Twelfth Biennial Cheese Industry Conference

Special thanks is given to Susan Rosinoff at the Forum Corporation of North America, Re: Total Quality Management (TQM Tool Kit) and Joe Choinski at the Concourse Corporation, Re: Statistical Methods for Improving Performance (Participant Manual) for giving permission to use selected material from the above mentioned publications.

With ever increasing competition in the world market place, it is imperative that any company involved in providing products and services offer their customers the highest quality at the lowest possible cost. The quality conscious consumer, having many demands on their limited income, are shopping for the best value for their family while, at the same time, company owners and stock holders expect the highest return on their investment. The answer to this two sided issue, as we all have come to realize, is quality. Quality pays! Quality pays by reducing or eliminating lost product, reruns, manufacturing costs, recalls and, in the final analysis, dissatisfied customers.

Quality can be defined as providing products and services that meet or exceed our customer's needs and expectations. Once we have determined what our customer's needs and expectations are, by sitting down and discussing it with them, we can then set our production targets to meet their specifications (See Exhibit A - Dimensions of Customer Expectations and Perceptions). **Specifications** is the key word. As we apply statistical processes or tools to control product quality, our goal will always be to identify the problems that prevent us from manufacturing products that meet established specifications, understand the processes that contribute to those problems, eliminate those problems and continuously improve the process.

Our goal is to manufacture all of our products to meet customers specifications and be as similar as possible. To do this, we must reduce variation in each step of our process. Variation is defined as: The differences that occur in products or processes. A process will always produce some amount of variation in the products it produces. In other words, no two products will ever be exactly the same. A little variation is okay -- it doesn't affect the quality of our products. Too much variation, however, can cause problems. It can lead to low - quality products. There are two kinds of variation: Common and Special cause. The variation that we expect in a process can be caused by a number of factors. These factors are called common causes of variation. Common causes affect variation very little. When something unusual happens to a process, too much variation can occur. The factors that cause excess variation are called special causes.(1)

Because of the necessary time limits given for this presentation, a lot of time will not be spent explaining the statistical formulas used as tools for reducing variation in our manufacturing procedures. This information is provided in many good statistical process control books, several of which are listed or referenced through out or at the end of this paper. Our time will be utilized discussing how statistical tools can be implemented to reduce variation in conjunction with The Process Improvement Cycle as designed by the Forum Corporation of North America (See Exhibit B - The Process Improvement Cycle).

I. Collect Information From Customers

- A. When we want to make decisions about improving work processes, we can't depend only on our hunches or experience to decide what should be changed. We need to collect facts, or data. Getting the facts can tell us if something is wrong in

the first place And, if something is wrong, collecting data can help us determine what and where the problem is.

B. Two Main Reasons for Collecting Data

1. **Identifying and solving problems:** When enough data have been collected, we can identify the cause of a problem, then work on solving the problem. Usually a problem is made up of a series of smaller problems. We attack a big problem by collecting data, identifying smaller areas to focus on, and collecting data in those areas. When the small problems have been corrected, we go back and collect data on the big problem again to see if our corrections had any effect. Then we begin the process again. Identifying and solving problems is a continual cycle that lets us enhance the quality of our products.
2. **Monitoring and Modifying a Process:** The second reason we collect data is to monitor the ongoing quality of a process. Remember that a process is a set of steps we follow to accomplish our tasks. We collect data at each step to make sure that the specifications for that step are being met. Specifications, or specs, are the limits of acceptable variation set by the industry or the company. By monitoring each step of a process, we can correct problems early.

C. Two Types of Data

1. **Countable Data:** Data that can be counted, such as the number of rejects produced by a part of the process. Countable data are always expressed in whole numbers (such as 6, 8, 450, 98 etc.)
2. **Measurable Data:** Data that can be measured, such as weight, length, time, temperature, and so on. Measurable data don't have to be whole number

D. How to Collect Accurate Data

1. Make sure the data being collected are what you need.
2. Make sure the measurement instrument is accurate and appropriate.
3. Make sure to include all the necessary information. In order to analyze the data, information that identifies the data must be included. (2)

E. Tools Used to Collect Data

1. Fish Bone or Cause and Effect Diagrams (See Exhibit C)
2. Process Flow Charts (See Exhibit D)
3. Pareto Charts (See Exhibit E)
4. Check Sheets (See Exhibit F)

II. Convert Information Into Measures: Often, after we've gone to the trouble of collecting data, we don't know quite what the data tells us. Pages and pages of data collection sheets can look like jumbled sets of numbers. When data are arranged in a clear

and meaningful manner, however, patterns and relationships emerge. These patterns and relationships give us information that wasn't easy to see in the data alone.(3)

A. Tools for Converting Data Into Measures

1. Control Charts: Because variation is always present in our work processes, it's important to monitor our processes on an ongoing basis. Monitoring also helps us keep a system under control. We can detect problems before they become serious. And, when we change a process, we can see whether the change improved the process or made it worse. A control chart is a line chart that shows the variation in a process. The two charts that are used for measurable data are x-bar and R charts. X-bar charts are used to monitor the variation in the averages of the measurements taken for each sample. R Charts are used to monitor the variation in the ranges of the measurements taken for each sample. (4) (See Exhibits F, G and H)
2. Histograms: A histogram is a bar chart that shows measurable data, such as height, weight, or time. A histogram shows us variation, or how the measurements we take are distributed. The bars of a histogram are called cells. All cells in a histogram are the same width. The number of measurements that fall into a cell can also be referred to as the cell's frequency. Because the height of a cell relates to frequency, we can tell where most of the measurements fall by looking at the tallest cell. Looking at all the histogram's cells gives us a picture of the variation in the data. This picture is called the frequency distribution. (5) Two important calculations that are important when making and interpreting control charts, histograms and determining process capability are: 1) calculating the mean and 2) calculating standard deviation (See Exhibits I and J)

B. Tools for Determining Process Capability

1. Process capability analysis (See Exhibit K)
2. CP and CpK: If we are to truly progress toward reducing process variation, we must target the processes correctly. An excellent method that will give us a measure of process targeting is CP and CpK. As a company implements this method, it may be necessary to first develop this process capability information from finished product analysis. Although we eventually want to move this measure to the in-process analytical, the tool will give us a picture of the current state of capability and the success of final centering of the process. (See Exhibit L)

III. Understand the Current Process: Once data has been collected from customers (internal and external) and converted to measures, the team involved in solving the problem or improving the process will then examine each step in the process to determine where and which problem to start eliminating first. Several good tools used to help understand the process are:

- A. Process Flow Diagrams: A process flow diagram is a picture of the steps used in making a product. (Reexamine Exhibit D)
- B. Fish Bone or Cause and Effect Diagrams. (Reexamine Exhibit C)

- C. Multi voting (See Exhibit M)
- D. Five (5) Why's (See Exhibit N)

- IV. **Design Improved Process:** After completing steps I - III, we now understand our process. We are now ready to design an improved process. We have determined, at this point in each step of the process, if we are in statistical control or not. We will assume here that we have eliminated all sources of special cause variation. If our common cause variation is in control and provides results that meet customer expectations (specifications), we continue to run as is using control charts to monitor our process. If our process capability (common cause variation) is in control but does not meet customer specifications, we must redesign our process. This may require new or improved processing equipment, improved instrumentation and testing procedures and employee training.
- V. **Measure:** After changes have been made to improve the process, we continue to measure using statistical tools to ensure that the changes we have made do, in fact, improve our process. Do our products meet customer expectation? If they do not, we must reexamine our processes continuing to use The Process Improvement Cycle in conjunction with statistical tools until we meet those needs.
- VI. **Standardization:** Once we have achieved the goal of having process capability meet or exceed customer expectations, is our work complete? No. We continue to focus on standardizing each step in the process while always looking for better ways to improve. Now we start over using The Process Improvement Cycle as a guide in conjunction with statistical tools to further refine our operations thus decreasing costs and improving customer satisfaction even more

EXHIBIT A

DIMENSIONS OF CUSTOMER EXPECTATIONS AND PERCEPTIONS

- What** A list that categorizes customers' expectations and perceptions and provides a framework for categorizing information from customers.
- When** Use the dimensions of customer expectations and perceptions to:
- identify your customers' expectations about outputs of your work unit
 - identify the specific gaps between customer expectations and perceptions in order to prioritize opportunities for improvement (Step 1)
 - set up the exercise of taking the customer's place
- Who** Managers and employees.
- How** To use the dimensions of customer expectations and perceptions, review the list below¹ and select those categories that are applicable to your customers' expectations and perceptions. Add any dimensions that you feel pertain specifically to your customers' situations. (Note: Throughout this list, the term "output" is used to represent your products, services, or information.)
- **Performance:** how well the output does what it is supposed to do.
 - **Tangibles:** the physical aspects of your output.
 - **Features:** the characteristics of your output that exceed the output's basic functions.
 - **Reliability:** the ability of the output (and its provider) to function as promised.
 - **Conformance:** the degree to which an output meets design and operation specifications.
 - **Durability:** how long your output lasts.
 - **Perceived quality:** the relative worth of your output in the eyes of customers.
 - **Serviceability:** how easy it is for you or the customer to fix your output with minimal downtime or cost.
 - **Assurance:** the knowledge and courtesy of the work unit's employees and their ability to elicit trust and confidence.
 - **Empathy:** the demonstration of caring and individualized attention to customers.
 - **Responsiveness:** the willingness and readiness of employees to help customers and provide prompt service.

¹ The 11 dimensions of customer expectations listed in this section are based on work by D.A. Garvin (Harvard Business School) on factors of product quality and A. Parasuraman, V.A. Zeithaml, and L.L. Berry (Texas A&M) on factors of service quality.

The Process Improvement Cycle

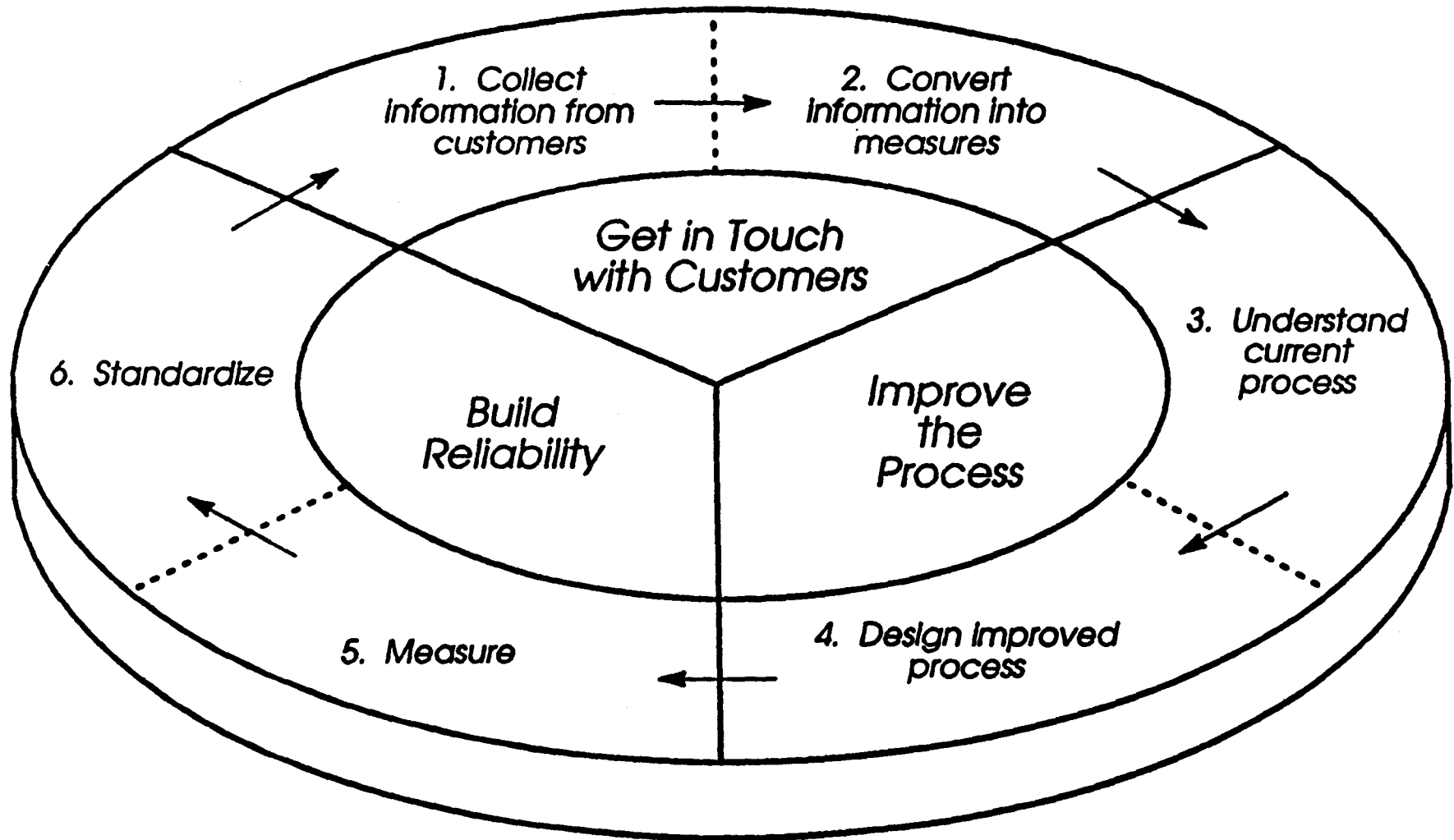


EXHIBIT C

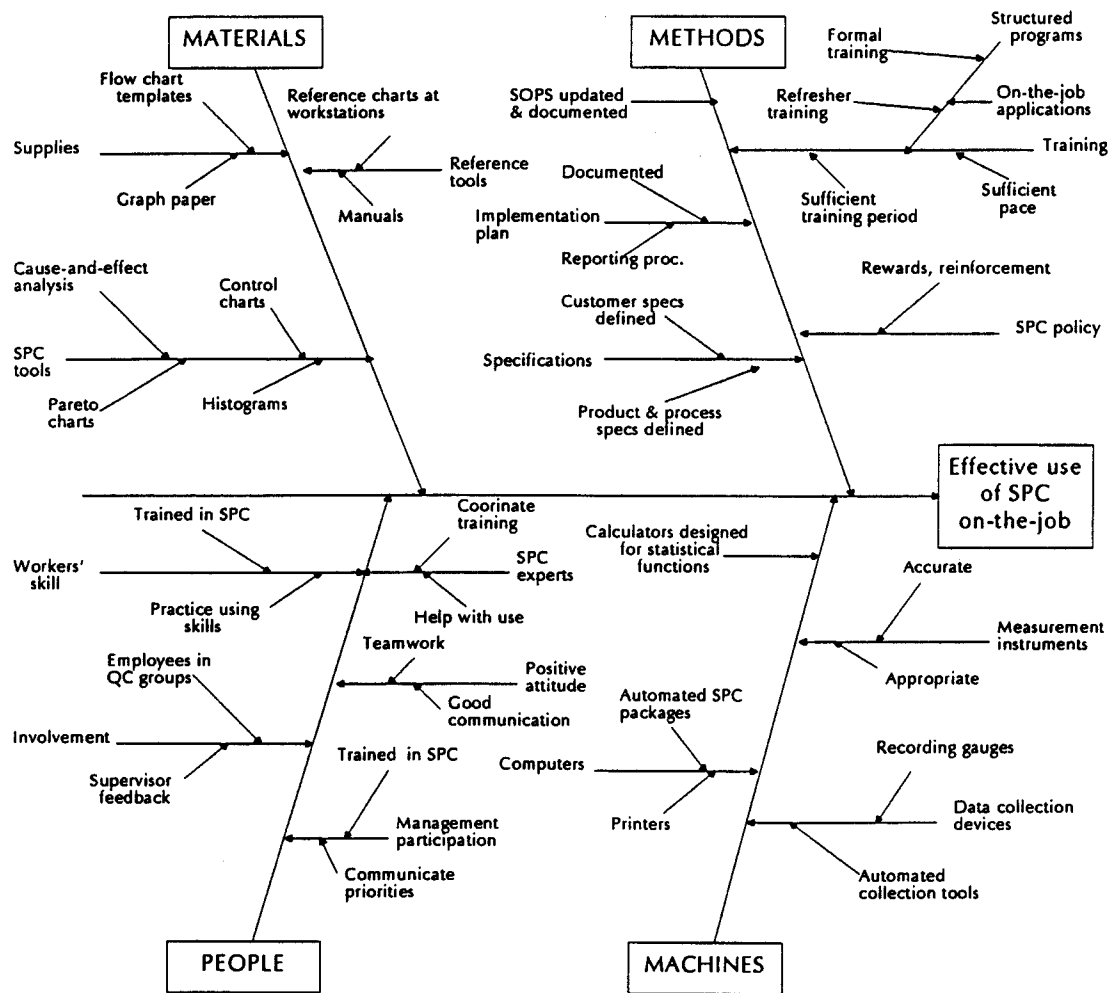


Figure 1. Cause-and-Effect Diagram of Effective SPC Use

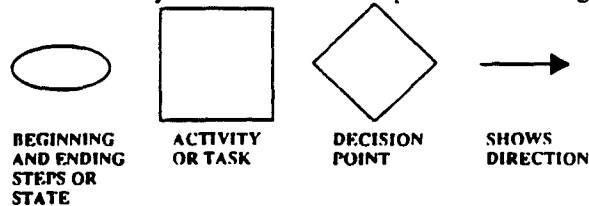
Cause-and-effect Analysis

FLOWCHART

What

A graphic representation of a process, enabling all the steps or activities to be seen in order, and the relationships between steps in a process to be visualized.

Flowcharts use symbols that have specific meanings:



When

Use a flowchart to:

- analyze the current process (Step 3)
- map inputs, outputs, flow of activities, and measures of the current process (Step 3)
- design an improved process (Step 4) by mapping an improved process flow, including measures

Use two flowcharts to:

- compare and contrast a map of the current process with a map of the desired process to examine where breakdowns may presently be occurring

Who

Managers and employees.

How

To create a flowchart:

- Identify the beginning and end points of a process and circle them.
- Use arrows to indicate flow of work from one activity to the next. Typically, only one arrow comes from an activity box, which is represented by a square.
- Use a diamond to indicate a decision point. A diamond will have more than one arrow coming from it. Typically, a "yes" decision flows vertically and a "no" decision flows horizontally.

Results

A flowchart provides:

- a map of the current process or an improved process
- an awareness of all the functions involved, tasks, handoffs, inputs, and outputs, as well as decisions in a process
- an opportunity to examine where breakdowns may be occurring in the current process

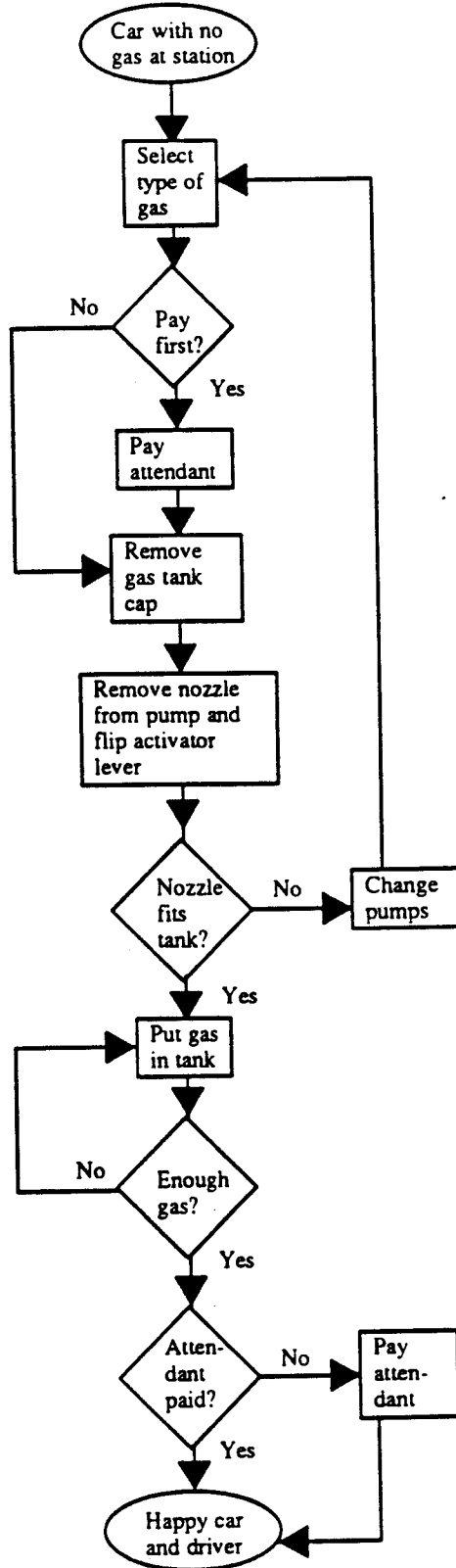
For Further Information

Process Quality Management and Improvement. Indianapolis, Ind.: AT&T, 1989.

Juran, J.M. *Juran on Planning for Quality.* New York, N.Y.: The Free Press, 1988.

FLOWCHART (cont.)

Example: FILLING UP



PARETO CHART

- What** A form of vertical bar graph that helps to identify problems by the frequency of their occurrence in a given process. As a graphic display, the Pareto chart draws attention to, and enlists cooperation in, making improvements. The Pareto chart is effective because of its ability to graphically demonstrate how seemingly small matters can cause big problems, with taller bars representing more significant problems and the shorter bars, the less significant problems.
- When** Use Pareto charts to:
- categorize data to identify improvement opportunities (Step 3)
 - rank improvement opportunities and set objectives (Step 3)
 - show the relative importance of problems (Step 4)
 - assess conformity to customer requirements
 - improve process quality
- Who** Managers, often in collaboration with, and input from, employees.
- How** To construct a Pareto chart:
- Select problems to be compared and rank ordered by nominal group technique or by examining existing data (such as previous quality reports).
 - Select the unit of measurement that appropriately quantifies problems to be assessed.
 - Determine how much time to allow for data collection.
 - Draw horizontal and vertical axes on graph paper (see example, page 44). On the left-hand vertical axis, label the measurement values in equal increments.
 - Draw in the bars, with the height of each bar determined by the corresponding value on the vertical axis. Each bar should have the same width and be drawn in contact with the bars next to it.
 - Label each bar below the horizontal axis according to the problem it represents.
 - Reorder the bars, going from left to right in order of decreasing frequency or cost.
 - Label the vertical axis on the right-hand side of the graph as the cumulative percent of the total distribution.
 - Plot a percentage line showing the cumulative total reached with the addition of each problem category. Once all problems have been represented, the total distribution should be 100 percent.
 - Title the graph and write the source of the data on which the graph is based; with quality control, the source of the data must be clear. Also, include all pertinent facts that will help define the parameters of observation.
 - Compare the frequency or cost of each problem category relative to all others.

PARETO CHART (cont.)

Results

A Pareto chart provides:

- an analysis, ideally, of how approximately 20 percent of the problems can cause 80 percent of the defects or costs
- a graphic demonstration of how much damage can be caused by a few vital errors
- a first step in making improvements

Note: It is most worthwhile to work first on whatever problem (or cause) is represented by the tallest bar on the Pareto chart. Remember that the most frequent problems are not always the most costly.

For Further Information

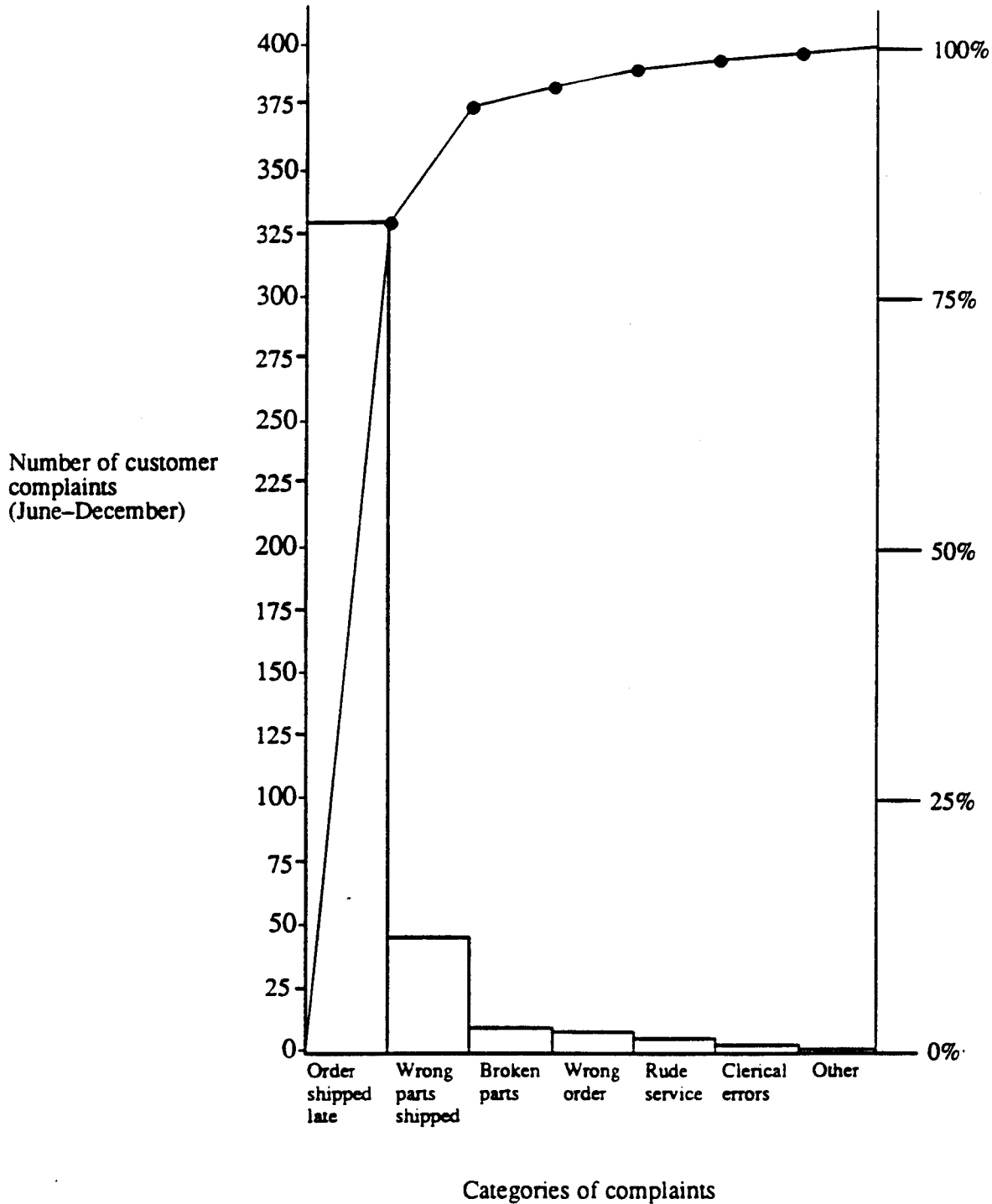
Ishikawa, Kaoru. *Guide to Quality Control*. White Plains, N.Y.: Unipub, 1988.

Scherkenbach, William. *The Deming Route to Quality and Productivity*. Washington, D.C.: CEEP, 1988.

EXHIBIT E

PARETO CHART (cont.)

Example: COST TO CORRECT CUSTOMER COMPLAINTS



Source: Information collected by Quality Assurance, June-December.

VARIABLE DATA

EXHIBIT

PRODUCT		CHARACTERISTIC	PROCESS TARGET	SPECIFICATION	ZERO EQUALS
OPERATOR		MACHINE	MEASURING INSTRUMENT	MEASUREMENT UNIT	
DATE					
TIME					
SAMPLE MEASUREMENTS	X ₁				
	X ₂				
	X ₃				
	X ₄				
	X ₅				
SUM					
\bar{x}					
R					
X					
R					

COMMENTS

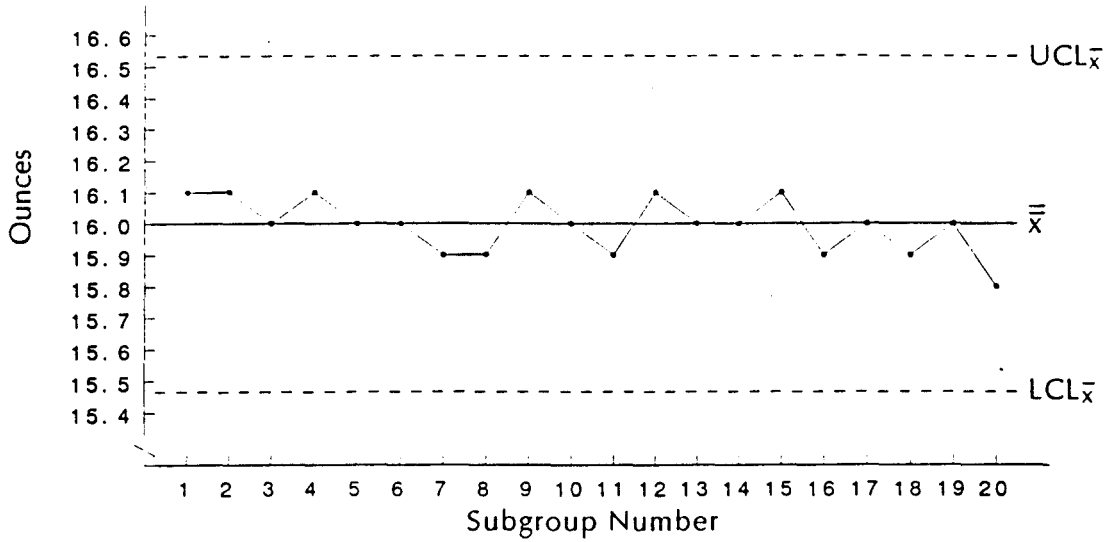
CONTROL CHART 

What	<p>A statistical method for understanding how a process functions and for monitoring variations in the process. Specifically, control charts are used to determine whether a process is, statistically speaking, in or out of control. They feature upper and lower control limits that are calculated using prescribed formulas; then, the averages taken from sample lots are plotted on the chart to determine the distribution of the data. Control charts monitor the distribution of the data over time, signal out-of-control situations, and are effective as a "real-time" tool. Note: Control charts only detect changes or variation in the process; alone, they do not determine the cause of the variation. An in-control process does not mean that the product or service produced will meet the requirements or specifications. A control chart can help you determine if the process is out of control, so you may take the necessary corrective action.</p>
When	<p>Use control charts to:</p> <ul style="list-style-type: none">• analyze and evaluate variation in the process• monitor the variation in the process in order to reduce it
Who	<p>The people who work in the process.</p>
How	<p>To use control charts:</p> <ul style="list-style-type: none">• Collect sample data in subgroups according to the sampling plan. For variable data, calculate the average and the range and plot on an X-bar and R chart. (There are other charts used for variable data and different charts used for attribute data.) <p>If the process is in control and capable, then only common cause variation exists. If the process is out of control, the variation is most likely from special causes. Elimination of those causes can improve the process. An out of control process is a signal that something different is occurring.</p>
Results	<p>A control chart can be used to:</p> <ul style="list-style-type: none">• make decisions based on data• help people affect a process and improve it• provide a means to determine if a process is out of control, signaling the user to take such actions as investigating, eliminating, or continuing to monitor the process
For Further Information	<p>Harrington, H. James. <i>The Improvement Process: How America's Leading Companies Improve Quality</i>. New York, N.Y.: McGraw-Hill, 1987.</p> <p>Ishikawa, Kaoru. <i>Guide to Quality Control</i>. White Plains, N.Y.: Unipub, 1988.</p> <p>Scherkenbach, William. <i>The Deming Route to Quality and Productivity</i>. Washington, D.C.: CEEP, 1988.</p>

EXHIBIT H

Wheat Bread Loaf Weight
 Batch #12 4/26/XX
 Prepared by: AJW

\bar{x} Chart



R Chart

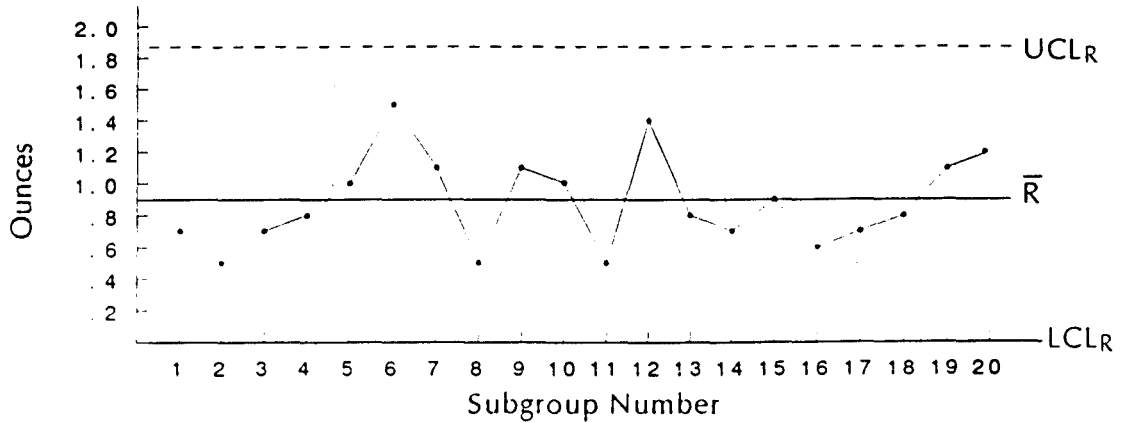


Figure 1. \bar{x} -R Charts for Loaf Weights

Both the averages of groups of data and their ranges (or reading-to-reading variability) are important when monitoring a process. Therefore, \bar{x} charts and R charts are almost always used together. For this reason, they are commonly referred to as \bar{x} -R charts (pronounced "x bar R charts").

CALCULATING THE MEAN

Mean—the average value of a set of data. The average value is the *mathematical center*. The mean is represented by the symbol \bar{x} (pronounced "x bar").

$$\bar{x} = \frac{\text{Total of measurements}}{\text{number of measurements}}$$

Importance of Calculating the Mean

The mean pinpoints the center of the data. For example, figure 8 shows the loaf weight histogram with the mean added. We can see that the line falls in the center of the distribution.

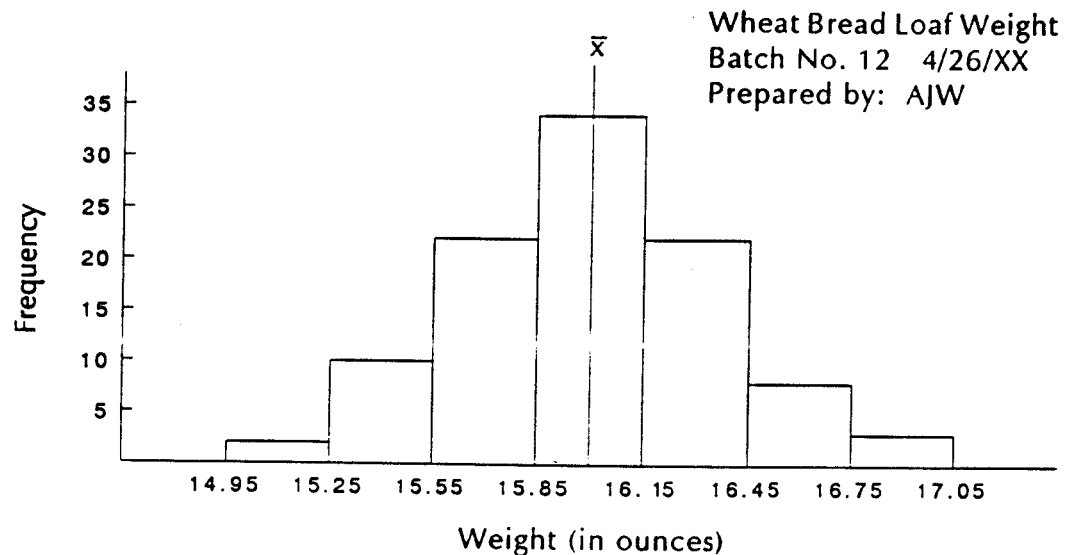


Figure 8. The Mean of the Loaf Weight Data

We can use the mean as a point of reference. By comparing each measurement to the mean, we can see how far above or below the average the measurement is. This, in turn, tells us how precise the process is. The closer the measurements are to the mean, the less variation there is in the process.

Notes:

APPENDIX: CALCULATING STANDARD DEVIATION

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

The formula used to calculate standard deviation is as follows:

- σ = standard deviation
- Σ = the sum of
- x = the individual values
- \bar{x} = mean
- n = the number of values

Suppose we want to calculate the standard deviation of the following data.

2.7, 2.4, 2.9, 2.5, 2.7, 3.3, 2.6, 3.0, 2.4, 3.5

We can do this by using the following five steps.

1. We find the mean (\bar{x}) of the data by adding the data values and dividing by the number of values ($28 \div 10 = 2.8$).
2. We construct a worksheet to calculate each value minus the mean squared $(x - \bar{x})^2$

x	$(x - \bar{x})$	$(x - \bar{x})^2$
2.7	-.1	.01
2.4	-.4	.16
2.9	.1	.01
2.5	-.3	.09
2.7	-.1	.01
3.3	.5	.25
2.6	-.2	.04
3.0	.2	.04
2.4	-.4	.16
3.5	.7	.49

In the x column, we simply list the individual data values. To find $(x - \bar{x})$ for each value, we subtract the mean ($\bar{x} = 2.8$) from the value. For example, the calculation of $(x - \bar{x})$ for the first value is: $2.7 - 2.8 = -.1$.

EXHIBIT J

To find $(x - \bar{x})^2$, we multiply $(x - \bar{x})$ by itself. Again, for the first value, the calculation is: $-.1 \times -.1 = .01$.

We continue this way until we have filled in the worksheet.

3. Once we have found $(x - \bar{x})^2$ for each value, we must add the values in the $(x - \bar{x})^2$ column to find $\Sigma(x - \bar{x})^2$. In this example, the sum is 1.26.
4. We divide the sum found in step 3 by $n - 1$. n is the number of data values. In this example, $n = 10$. So $n - 1 = 9$. Dividing 1.26 by 9, we get .14.
5. In the last step, we take the square root of .14, which is .374 (rounded to the nearest thousandth). $\sigma = .374$.

Importance of Standard Deviation Values

Figure 9 shows the loaf weight histogram with the mean and standard deviation values added.

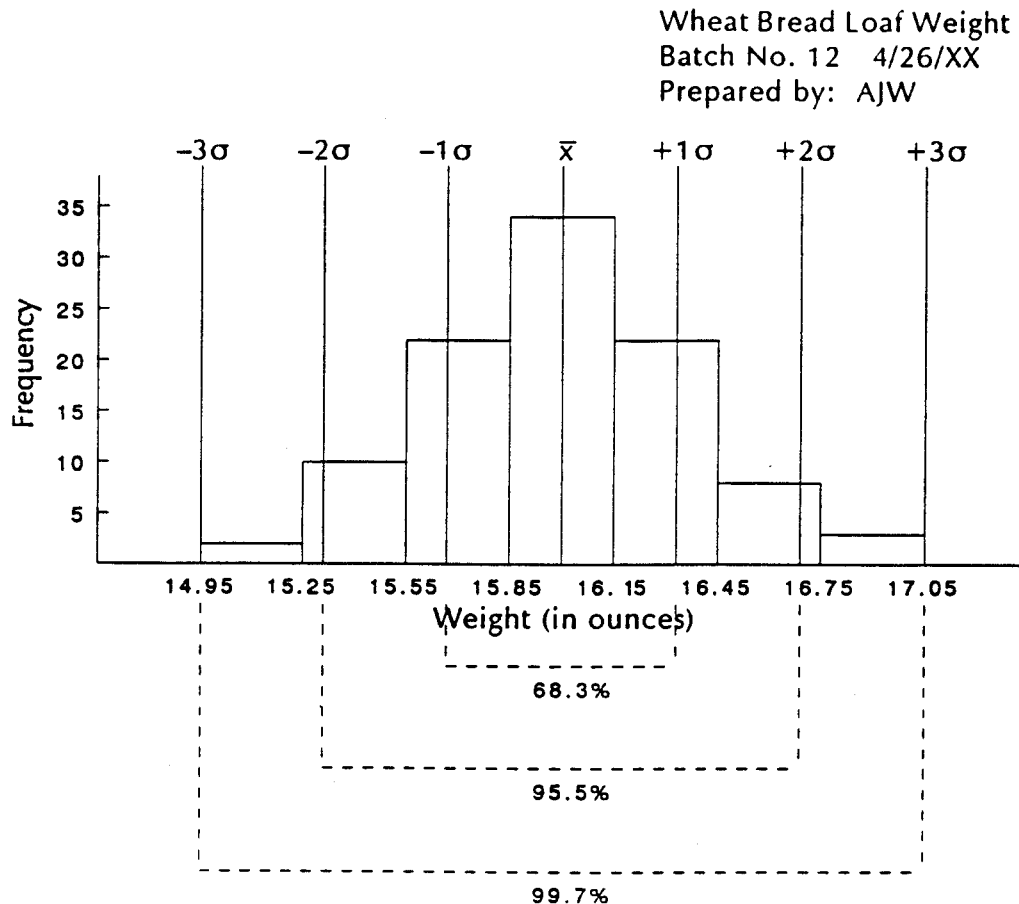


Figure 9. Standard Deviation Values for the Bread Loaf Data

The pattern shown by the loaf weight histogram is always true for a normal distribution. Therefore, we can predict the approximate percentage of data that will usually fall between the mean and each standard deviation value for any normal distribution.

For any normal distribution, approximately 68.3 percent of the data will fall between -1σ and $+1\sigma$. This is over two-thirds of the data! Approximately 95.5 percent of the data will fall between -2σ and $+2\sigma$, and approximately 99.7 percent of the data will fall between -3σ and $+3\sigma$.

EXHIBIT K

PROCESS CAPABILITY ANALYSIS

What	An analysis which helps determine the extent to which the output of a process, namely, the given product, service, or information, meets or exceeds the expectations and requirements of the customer. Capability analysis compares the natural process limits with the customer's expectations or engineering tolerances to see if the process is producing output to specification; in other words, capability analysis determines if the natural behavior of the process is producing an output that meets the requirements of the customer.
When	Use capability analysis to: <ul style="list-style-type: none">• analyze and evaluate the capability of the current process (Step 3)• continuously improve the process (Step 4)• assess whether a process is meeting customer specifications <p>Note: An analysis of capability is only performed after it has been determined it has been determined that the process is statistically in control.</p>
Who	Managers in collaboration with employees.
How	To use capability analysis: <ul style="list-style-type: none">• Check to see if the process is statistically in control since capability analysis is otherwise not valid. (When a process is statistically in control, the average is plus or minus 3 standard deviations, or within 6 sigma. This simply means that the distribution is assumed normal, centered around the mean, and is consistent.)• Determine capability by indexes; these indexes are ratios which can be calculated mathematically and indicate the ability of a process to produce outputs that conform to a given specification. For example, consider the process of manufacturing dowels for a furniture maker, the customer. The customer specifications call for dowels 8 inches long and 1 inch in diameter, while the process consistently produces dowels that are 7-1/2 inches long and 3/4 inch in diameter. Although the process is consistent and in control, it is not capable because the dowels do not meet the customer's specifications. The process must be changed so that it will consistently produce dowels that are 8 inches long and 1 inch in diameter.
Results	A capability analysis provides: <ul style="list-style-type: none">• a data-based determination of whether a process is meeting or is capable of meeting (or exceeding) customer expectations
For Further Information	Ishikawa, Kaoru. <i>Guide to Quality Control</i> . White Plains, N.Y.: Unipub, 1988. Juran, J.M. <i>Juran on Planning for Quality</i> . New York, N.Y.: The Free Press, 1988.

CP/CpK

EXHIBIT L

We intend to look at key products at all plants and measure the plant's ability to reduce variation and center the processes. To this end we will be using capability indices commonly known as CP and CpK. The following is a review of the theory of these measures and a couple practical examples.

The *process capability (CP)* is a measure of the dispersion of data about the mean and is described in relation to the specs or tolerances for the product. It in fact is a ratio of the tolerance to 6 sigma for the process.

$$CP = \frac{\text{Tolerance}}{6\sigma}$$

Tolerance = Specification Width, i.e. $\pm 0.5\%$

σ = Process Sigma (6 σ describes the 99.97% confidence interval)

The significance of a CP is described below:

1. $CP < 1$ indicates that the tolerance is smaller than the 6 sigma of the process. That is, the process does not fit in the specs.
2. $CP > 1$ indicates that the tolerance is larger than the process sigma and therefore the process fits within the specifications. Typically, a CP of 1.33 is considered ideal. This value equates to ± 4 sigma range for the process to work in. In general, the greater the CP number the better.

The CP as stated measures the data spread but it does not address whether the process is centered. A process could have a CP of 1.33 and still have significant product out of specification due to the fact that one or both tails of the distribution could be outside the spec limits. The tool used to identify the centering of the process is the *CpK*. Actually, the CpK is a measure of dispersion as well as centering of the process.

$$CpK = \frac{(USL - \text{Mean})}{3\sigma} \text{ or } \frac{(\text{Mean} - LSL)}{3\sigma}$$

The smaller of the resulting calculations above is taken as the CpK.

USL = Upper Specification Limit, i.e. $48 \pm 0.5 = 48.5$

LSL = Lower Specification Limit, i.e. $48 \pm 0.5 = 47.5$

Mean = Mean (average) of Process data, i.e. mean of 48.14% on a target of 48.0%

The significance of the CpK is described below:

1. $CpK > 1$ indicates the spread of data (6 sigma) falls completely within the spec limits.

EXHIBIT L

As in the CP, the larger the number the better. A CpK of 1.33 means that the difference between the mean and the spec limit (*closest limit*) is equal to 4 sigma rather than 3 sigma.

2. CpK = 1 indicates that one tail of the 6 sigma spread lies on a spec limit.
3. CpK = 0 - 1 indicates that some portion of the 6 sigma spread falls outside the spec limits.
4. CpK with a *negative* value indicates that the mean of the process lies *outside* the spec limits.

Cp and CpK will both be used in evaluating process performance. When a process hits target exactly, CP = CpK. This then is a measure of how much better the CpK could be if the process was centered on target.

CP speaks to whether the the data distribution could fit in the spec limits if centered on target while the CpK addresses whether the distribtion does.

Please note that X and MR charts of the data should indicate that you are substantially in control for these measures to be as useful as they should be. Such should be the case if you are not routinely experiencing out-of-standards.

EXHIBIT L

IV.

Specification = $69.0 \pm 0.5\%$
Process SD = 0.21
Mean = 69.2

$$CP = \frac{1.0}{1.26} = 0.79$$

The CP is < 1.0 , therefore the process does not fit the specs. The process is not capable.

$$CpK = \frac{69.5 - 69.2}{0.63} = 0.48$$

$$CpK = \frac{69.2 - 68.5}{0.63} = 1.11$$

The value chosen is 0.48 since it is smaller. A value between 0 - 1 indicates that some portion of the 6 sigma data spread is beyond a spec limit.

III.

EXHIBIT L

Specification = $48 \pm 0.8\%$
Process SD = 0.15
Mean = 48.14%

$$CP = \frac{\text{Tolerance}}{6\sigma}$$

$$CP = \frac{2 \times 0.8}{6 \times 0.15} = \frac{1.6}{0.9} = 1.78$$

Process is capable.

$$CpK = \frac{(USL - \text{Mean})}{3\sigma} \text{ or } \frac{(\text{Mean} - LSL)}{3\sigma}$$

$$CpK = \frac{48.8 - 48.14}{0.45} = 1.79$$

$$CpK = \frac{48.14 - 47.2}{0.45} = 2.09$$

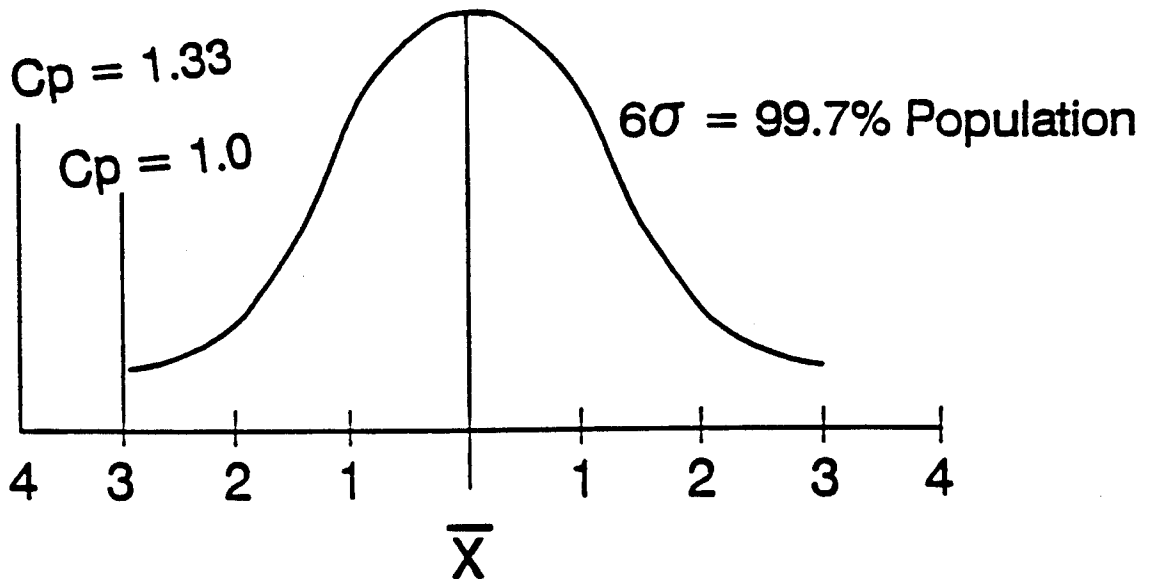
The 1.79 value is chosen as it is smaller. It is > 1.0 and therefore the process fits within the specifications.

Measures of Conformance

Spread:

- Variance ($\text{Var} = \sigma^2$)
- Standard Deviation = σ
- **Cp Index = $\frac{\text{Specification Range}}{6\sigma}$**

Normal Distribution



- When the capability (Spread) is exactly equal to the width of the Specification, the $C_p = 1.0$.

Goal for C_p is 1.33

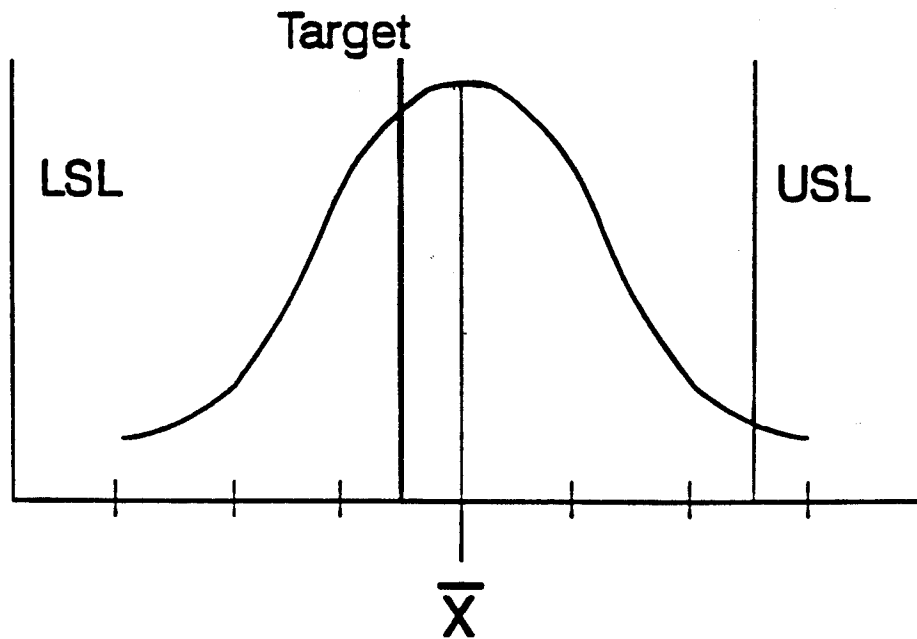
Measures of Conformance

Centering:

- Mean (\bar{X} = Average)
- Median
- Mode
- Target

$$CpK = \frac{(USL - \text{Mean})}{3\sigma} \text{ or } \frac{(\text{Mean} - LSL)}{3\sigma}$$

(The smaller of the two values is the CpK)



- | | |
|---------------|-----------------------------------|
| $CpK > 1$: | within spec limits |
| $CpK = 1$: | one end is on the spec limit |
| $CpK = 0-1$: | some portion is out of spec |
| $CpK < 0$: | the \bar{X} is outside the spec |

Goal for CpK is to be > 1

What Cp and CpK Can Do for You

- ▶ Serves as a tool to help identify and prioritize issues with analytical specifications and Targets.
- ▶ Cp and CpK can be tracked to measure improvement.
- ▶ Easy to remember reference for capability and target versus average.

EXHIBIT M

MULTIVOTING

- What** A structured method of reducing a long list of items to a manageable few. Multivoting provides a way to reach consensus without lengthy debate.
- When** Use multivoting when you need to:
- narrow the list of potential issues or problems that the team could work to improve
 - focus the team's effort on the critical few
 - arrive at consensus within a team that is not used to working together
- Who** Managers, employees, and improvement teams.
- How** To use the multivoting technique:
- Brainstorm a list of issues or problems. (When starting to design an improved process, brainstorm possible solutions or process improvement ideas instead.)
 - Combine similar items only if the group agrees that they are redundant.
 - Assign a letter (not a number) to each of the remaining items.
 - Establish the number of votes allotted to each team member. (The number of votes per team member equals 20% of the total number of items. If there are forty items, each member is allotted eight votes.)
 - If necessary, establish the guidelines or criteria by which members will cast their ballots. Make sure all team members agree upon these criteria.
 - In the first round of voting, each member selects items by casting his or her allotted number of ballots. Emphasis is indicated by casting more than one vote per item. Voting is completed in silence.
 - Tally the total number of votes received for each item. The total number of votes should equal the number of members times the number of votes allotted per person.
 - Reduce the list of items by eliminating those items with the fewest votes.
 - Reduce the list to two or three items by casting as many rounds of votes as are necessary. Each member has the same number of votes as in the first round. (Do not reduce the list down to one item. Instead, use a decision matrix to make the team's final selection.)
- Results** Multivoting provides:
- a focus for the team's effort
 - a way to engage everyone on the team
 - a method of reaching unspoken agreement without verbal conflict
- For Further Information** Scholtes, Peter R.. *The TEAM Handbook*. Madison, WI.: Joiner Associates, Inc., 1988.

5 WHY'S

- What** An analytic tool to aid an improvement team in exploring the relationship between a problem and its causes.
- When** Use the 5 Why's when you need to:
- identify the root cause of a problem (Step 3)
 - create a cause-and-effect diagram and are exploring deeper causes of a problem
 - explore a causal relationship more deeply
- Who** Managers, employees, and improvement teams.
- How** To use the 5 Why's:
- Identify the problem statement. Ask "Why is that happening?" (When working individually, simply complete the attached workchart. When working with a team, transfer the workchart to a flipchart or overhead.)
 - Continue to ask "why" until you are certain you have gone as far as you are able in identifying a possible root cause.
 - Complete the "possible solution" section of the workchart using brainstorming and multivoting.
- Results** The 5 Why's provide:
- a way for a group to challenge or explore beyond apparent or popular explanations for the causes of a problem.
 - a structured framework for exploring root causes.

References

Concourse Corporation

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References used: Statistical Methods for Improving Performance (Participant Manual): (1-5) and Exhibits C, H, I, J .

The Forum Corporation

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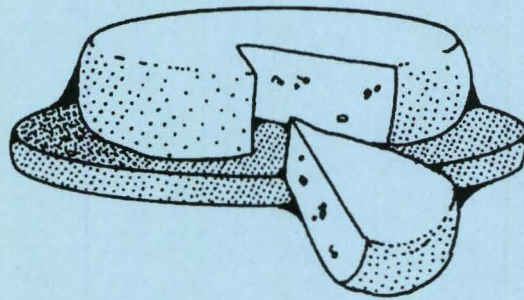
Boston, Mass. 02109

Phone: 1- 617-523-7300

Contact person: Susan Rosinoff

References used: Total Quality Management (TQM Tool Kit) Exhibits A , B, D, E, G, K, M, and N.

CHEDDAR CHEESE AS A SPECIALTY CHEESE?



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CHEDDAR CHEESE AS A SPECIALTY CHEESE?

Mark E. Johnson
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Stripped down to the basics, a specialty cheese is a cheese that is different from the ordinary. While we may not agree on this definition, there are several elements and perceptions that have been used to describe a specialty cheese:

1. Unique flavor or physical characteristic
2. Requires a special manufacturing process (artisans)
3. Manufactured in a specific geographical area
4. Specific milk is used
5. Specific bacterial cultures are used
6. Specific ingredients added (fruits, nuts, bacon bits)
7. Packaged in a unique manner
8. Shaped into a distinctive form
9. Imprinted with unique patterns
10. Value added
11. Produced in low volume

With over 2.35 billion pounds of Cheddar cheese produced in the US, Cheddar cheese hardly qualifies as a specialty cheese. Cheddar cheese in this sense, is defined in terms of composition and rather loose manufacturing parameters. "Specialty" implies an aura of uniqueness, peculiarity, or distinctiveness. But, don't we often conjure up an image of a particular Cheddar cheese just by name alone ? : Kraft Cracker Barrel, Wisconsin, Vermont, or English Cheddar. Anticipating a particular taste or quality, we buy on brand name, or descriptive terms such as mild, medium, aged, or sharp. So, are we not in fact calling attention to the uniqueness, the distinctive character of a "specialty cheese" ?

In addition, there is an increased interest in Cheddar cheese as an ingredient in prepared foods. This requires that Cheddar cheese be made with specialized manufacturing schedules and procedures in order to meet specific physical and organoleptic standards. Thus, manufacturers will make a "specialty" Cheddar cheese for a specific end use. A major challenge to cheese makers and scientists is the simultaneous attainment of both desirable flavor and body characteristics of Cheddar cheese for use in such specialized applications as a food ingredient.

How can "specialty" Cheddar cheeses be produced ?

There are tales of particular Cheddar cheese of a certain flavor or taste prescribed solely by the particular microbial flora contaminating the cheese processing area. Thus, Cheddar cheeses can be produced in the same geographical area of a similar milk supply and yet taste nothing alike. Certain flavors in cheese can indeed be a direct result of the metabolism of non-starter bacteria, including fruity, unclean, rancid and possibly sulphur (H₂S) containing flavor compounds. In balance these flavors may contribute to the distinctive and appealing Cheddar cheese taste, but out of balance they can become objectionable.

There are a number of ways Cheddar manufacturers have manipulated the development of flavor:

1. Time/temperature of storage
2. Starter
3. Addition of adjunct cultures
4. Addition of enzymes
5. All combinations
6. Manufacturing protocol

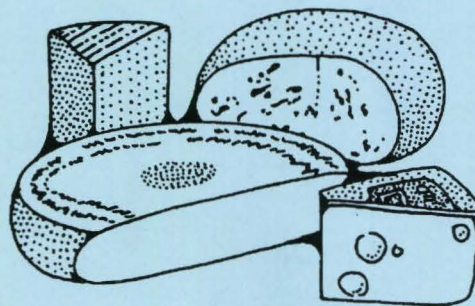
Similarly, cheese makers can manipulate the physical characteristics of cheese through the following:

1. Composition
2. pH
3. Proteolysis
4. Manufacturing protocol

But, describe Cheddar cheese flavor? Would two people from different parts of the world or even different parts of the country describe Cheddar cheese flavor in the same way? One of the problems in developing strategies to "enhance" or "improve" or alter the flavor of Cheddar cheese is that there is such a large diversity as to what constitutes acceptable and desirable Cheddar cheese flavor. Is it a single compound? One has not yet been identified. Is it a mixture of compounds? which ones? Undoubtedly, the answer rests with the individual interpretation of what Cheddar cheese is "supposed" to taste like. On the other hand, perhaps it is fortunate that we do not have a "standard" Cheddar cheese flavor and we revel in the diversity of Cheddar cheese flavors.

Over the past two decades the manufacture of Cheddar cheese has seen tremendous change. Major changes have taken place in the size, mechanization and speed of the cheese making process. Concomitant with these mechanistic changes is the pressure on the culture suppliers to provide reliable starters that meet the demands of fast paced, automated cheese making. These changes have forced some cheeses makers out of the business. For others, it has led to opportunity, built in part upon the perception of quality and added value of cheese produced by smaller processors when compared to the Cheddar cheese produced in larger, more automated plants. Whether perception or reality, is unimportant. What is important is that the small Cheddar cheese plant can now seize the opportunity to capitalize on consumers' perception of value. However, of utmost importance, the small volume Cheddar plant must still in fact produce a quality, value added product.

**FURTHER CHARACTERIZATION OF
GENETIC PROBE ISOLATED STRAINS
OF *LACTOCOCCUS CREMORIS* AND
THEIR USE IN CHEESEMAKING**



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**FURTHER CHARACTERIZATION OF GENETIC PROBE ISOLATED
STRAINS OF *LACTOCOCCUS CREMORIS* AND THEIR USE IN
CHEESEMAKING**

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At the conference two years ago we presented data describing the isolation, using 16S ribosomal RNA probes, of so-called "new" *Lactococcus lactis ssp. cremoris* strains from unpasteurized dairy products and raw milk. These samples were all from areas of the world where primitive milk handling practices such as hand-milking into open containers are still used. We have not been successful in attempts to isolate new *cremoris* strains from domestic raw milk or green plant extracts. Since our last report Brent Daniels has been characterizing these isolates and making cheese with some of them at the T.C.C.A. in Tillamook, OR, and Ena Urbach a postdoc in Dr. Steve Giovannoni's lab, has been sequencing the DNA encoding lactic dehydrogenase in these bacteria. The latter has been done to use this molecular taxonomy approach to learn how these findings would compare with the classical methods of identifying *Lc. cremoris* by phenotypic characterization. The ultimate goal of this research is to supply the dairy industry with new strains of *Lc. cremoris* with a different genetic background from currently used industrial strains. We also wanted to learn whether or not these strains would be of increased value for Cheddar cheesemaking from flavor and phage resistance standpoints. Findings on these points will be presented as well as data from Dr. Urbach's work suggesting that molecular approaches are of value in classifying *Lc. cremoris* strains at the subspecies level.

References

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3. Salama, M. S., W. E. Sandine, and S. J. Giovannoni. 1993. Isolation of *Lactococcus lactis* subsp. *cremoris* from nature by colony hybridization with rRNA probes. *Appl. Environ. Microbiol.* 59:3941-3945.
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Submitted: 7/15/96

**The *ldh* Phylogeny for Environmental Isolates of *Lactococcus lactis*
is Consistent with rRNA Genotypes, but not with Phenotypes**

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Running title: *Lactococcus lactis ldh* phylogeny

Keywords: *Lactococcus lactis*, molecular evolution, Cheddar cheese

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Table 1. Characteristics of environmental isolates.

Strain	Source	Growth at			Hydrolysis of Arginine	Phenotypic subspecies	Probe 68RCa	Coagulation of milk		Flavor development in milk	Reference
		40°C	4% NaCl	pH 9.2				15-18 hr	24hr		
112 ^a	Bosnia, cottage cheese sample 1	+	+	-	-	?	-	+	+	bitter, malty	27
1117M	Bosnia, cottage cheese sample 1	-	-	-	-	cremoris	+	-	-	bitter, off flavor	27
AM4	Bosnia, cottage cheese sample 2	-	-	+/-	-	?	+	+	+	bitter	
AM5	Bosnia, cottage cheese sample 2	-	-	-	-	cremoris	+	-	+	flat	27
AM12	Bosnia, cottage cheese sample 2	+	-	+/-	-	?	+	+	+	bitter, malty	27
BEN121	U.S., <i>Faseolus vulgaris</i>	+	+	+	+	lactis	-	-	+	bitter, malty	25, 27
BO32	Bosnia, raw milk	+	+	+	-	lactis	-	-	-	flat, off flavor	27
BO34	Bosnia, raw milk	+	+	+	-	lactis	-	-	+	bitter	
BO35	Bosnia, raw milk	+	+	+	-	lactis	-	-	+/-	bitter	
BO36	Bosnia, raw milk	+	+	+	-	lactis	-	-	+	bitter	
BO38	Bosnia, raw milk	+	+	+	-	lactis	-	-	+	bitter	
BO39	Bosnia, raw milk	+	+	+	-	lactis	-	-	+	bitter	
CO3	U.S., <i>Zea maize</i>	+	+	+	+	lactis	+	+	+	slightly malty	25, 27
CM1-3 ^b	China, raw milk sample 1	-	+	-	+	?	+	-	+	bitter	25, 26, 27
CM4-27 ^c	China, raw milk sample 4	+	-	+	+	?	+	+	+	slightly bitter	25, 26, 27
CM5-6	China, raw milk sample 5	+	-	-	+	?	+	+	+	acid, chalky	26, 27
FB1	U.S., bovine colostrum sample 2	+	+	+	+	lactis	-	-	+/-	clean, acid	25, 26, 27
FB62	U.S., <i>Rubis discolor</i>	+	+	+	+	lactis	-	-	+	clean, acid	25, 27
MS3	Morocco, raw milk	+/-	+/-	+/-	+	?	-	+/-	+	slightly bitter	27
MS5	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	slightly bitter	26, 27
MS7	Morocco, raw milk	-	-	-	+	cremoris	-	+/-	+	clean, acid	27
MS9	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS11	Morocco, raw milk	-	-	-	-	cremoris	-	+	+	clean, acid	25
MS13	Morocco, raw milk	-	-	-	+/-	?	+	-	+	clean, acid	
MS16	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	slightly bitter	
MS17	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	27
MS23	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS24	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS25	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS26	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS27	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS31	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS33	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS34	Morocco, raw milk	-	-	-	-	cremoris	+	-	+	bitter	
MS44	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS45	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS49	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS51	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS52	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	
MS53	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	clean, acid	25
MS55	Morocco, raw milk	-	-	-	-	cremoris	+	-	-	clean, acid	
MS58	Morocco, raw milk	-	-	-	-	cremoris	+	+	+	flat	25, 27
MS70 ^a	Morocco, raw milk	-	+	+	+	?	-	+	+	off flavor	25, 26
MSUA2	Ukraine, raw milk	-	+	-	-	?	+	-	-	off flavor	27
MSUA10	Ukraine, raw milk	+	-	-	-	?	+	-	+	bitter	27

^aPositive for probe 68RCa-binding in colony hybridization tests (25), but negative by hybridization to amplified 16S rRNA genes.

^bAs originally isolated, CM1-3 was negative for growth in 4% NaCl and for arginine hydrolysis. These properties changed after storage at -80°C (25).

^cAs originally isolated, CM4-27 was negative for growth at 40°C and at pH 9.2 and for arginine hydrolysis. These properties changed after storage at -80°C (25).

Table 2. Oligonucleotides used for PCR amplification and DNA sequencing.

Oligonucleotide	Sequence
LDHF1	ATG-GCT-GAT-AAA-CAA-CGT-A
LDHF2	GTT-GCT-GCT-AAC-CCA-GTT-GA
LDHF3	GGT-GCA-ACA-TTC-TAY-GGT-GT
LDHR1	TTA-GTT-TTT-AAC-TGC-AGA-AG
LDHR2	GTC-AAG-ATR-TCA-ACT-GGG-TT
LDHR3	ACA-CCR-TAG-AAT-GTT-GCA-CC

Table 3. Carbon substrate utilization profiles for *L. lactis* strains. All strains were negative for acid production from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, aldonitol, B methyl-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, α methyl-D-mannoside, α methyl-D-glucoside, melibiose, trehalose, melezitose, D-raffinose, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, 2 ceto-gluconate, and 5 ceto-gluconate. Strains are listed in the order they appear in Fig. 1.

strains	subsp. <i>cremoris</i>	Ribose	Galactose	D-Glucose	D-Fructose	D-Mannose	Mannitol	N acetyl glucosamine	Amygdalin	Arbutin	Esculin	Salicin	Cellobiose	Maltose	Lactose	Saccharose	Inulin	Starch	β Gentiobiose	Gluconate
AM-5			+	+	+	+		+								+				
CM4-27			+	+	+	+		+								+				
MS-5			+	+	+	+		+								+				
MS-9			+	+	+	+		+								+				
MS-13			+	+	+	+		+								+				
MS-16			+	+	+	+		+								+				
MS-17			+	+	+	+		+				+				+		+		
MS-23			+	+	+	+		+								+				
MS-24			+	+	+	+		+								+				
MS-25			+	+	+	+		+								+				
MS-26			+	+	+	+		+								+				+
MS-27			+	+	+	+		+								+				
MS-31			+	+	+	+		+				+				+				
MS-33			+	+	+	+		+			+					+				
MS-34			+	+	+	+		+	+	+	+	+	+		+	+	+		+	
MS-44			+	+	+	+		+								+				
MS-45			+	+	+	+		+								+				
MS-49			+	+	+	+		+								+				
MS-51		+	+	+	+	+		+		+	+	+	+	+	+	+	+	+		
MS-52			+	+	+	+		+								+				
MS-53			+	+	+	+		+								+				
MS-55			+	+	+	+		+			+					+				
MS-58		+	+	+	+	+		+		+			+	+	+		+			
ML1*				+	+	+		+								+				
MSUA2			+	+	+	+		+								+				
MSUA10		+	+	+	+	+		+			+			+	+		+			
1117M		+	+	+	+	+		+			+					+				
AM-4			+	+	+	+		+								+				
AM-12			+	+	+	+		+								+				
MG1363*		+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+
CO3		+	+	+	+	+	+	+					+	+	+		+	+	+	+
BO32		+	+	+	+	+	+	+		+	+	+	+	+	+		+	+		
CM1-3		+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	
CM5-6		+	+	+	+	+	+	+			+	+	+	+	+		+	+	+	
subsp. <i>lactis</i>																				
BO34		+	+	+	+	+		+		+	+	+		+	+		+	+		
BO35		+	+	+	+	+		+		+	+	+		+	+		+	+		
BO36		+	+	+	+	+		+	+	+	+	+	+	+	+		+	+		
BO38		+	+	+	+	+		+		+	+	+		+	+		+	+		
BO39		+	+	+	+	+		+			+	+		+	+		+	+		
FB1		+	+	+	+	+		+		+	+	+	+	+			+	+	+	
FB62		+	+	+	+	+		+		+	+	+	+	+			+	+	+	
MS-70		+	+	+	+	+		+	+	+	+	+	+	+			+	+	+	
112			+	+	+	+		+								+				
MS-3			+	+	+	+		+								+				
MS-7			+	+	+	+		+								+				
MS-11			+	+	+	+		+								+				
BEN121		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Laboratory strains derived from commercial starter cultures.

Table 4. Phage sensitivity profiles for environmental isolates of *Lactococcus lactis*. Ten isolates were insensitive to all phage preparations, and 118 phage preparations were ineffective against all *L. lactis* strains.

	CM4-27	CO3	FB1	FB62	MS70	BEN121
Quest international composite wheys						
Quebec composite		3				
Waterford Foods plaque-purified phage						
3		3			3	
5		3			3	
6		3			3	
7		2			2	
8		3			3	
9		3			3	
10	2	3			3	
11		3			3	
12		3			3	
13		3			3	
14		3			3	
15		3			3	
16		3			3	
17		1			2	
19						2
20		1				2
21						2
23						2
24		1				2
26						2
Marshall Products phage-positive whey						
1				1		
4	1					
6				3		
7				1		
10		3				
11		2		1		
12		2				
13				1		
14				1		
18		2				
21	1					
26		2				
27	1	3	2			
28		2				
29		2				
31	1			1		
39	1	3	1	1		
40		3	1			
41		1				
44				2		
45		2				
46		2				
47		3				
49		2				
51		1				
52		1				
55		1				
57		2				
60		1				
63		1				
64		3				
68		3				
plaque-purified phage						
SPI 13				1		
SPI 18		1				
SPI 23				1		
SPI 24		3	1		1	
SPI 34		2		1		
SPI 38		1				

FIGURE LEGENDS

1

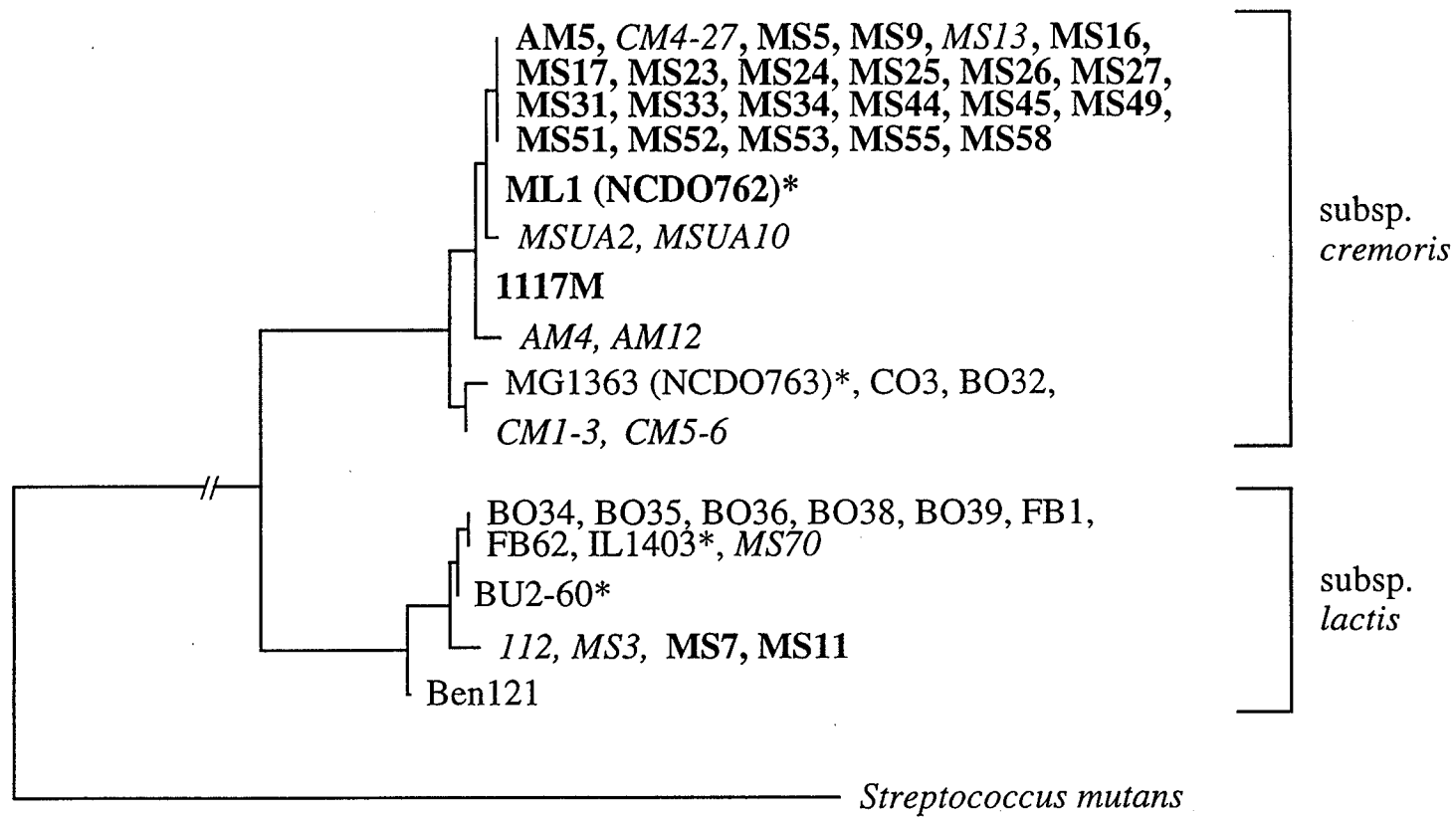
2

3 **Figure 1.** A neighbor-joining phylogenetic tree inferred from *L. lactate* dehydrogenase
4 gene sequences. 938 nucleotide positions were used in the analysis. The phylogenetic
5 relationships shown here correlated exactly with those obtained by ribosomal RNA
6 sequencing and strain identification by hybridization to *L. lactis* and subsp. *cremoris*-
7 specific oligonucleotide probes. **Bold text:** phenotypically subsp. *cremoris*. Plain text:
8 phenotypically subsp. *lactis*. *Italic text:* phenotypic subsp. indeterminate. Asterisks (*)
9 indicate laboratory strains derived from commercial starter cultures.

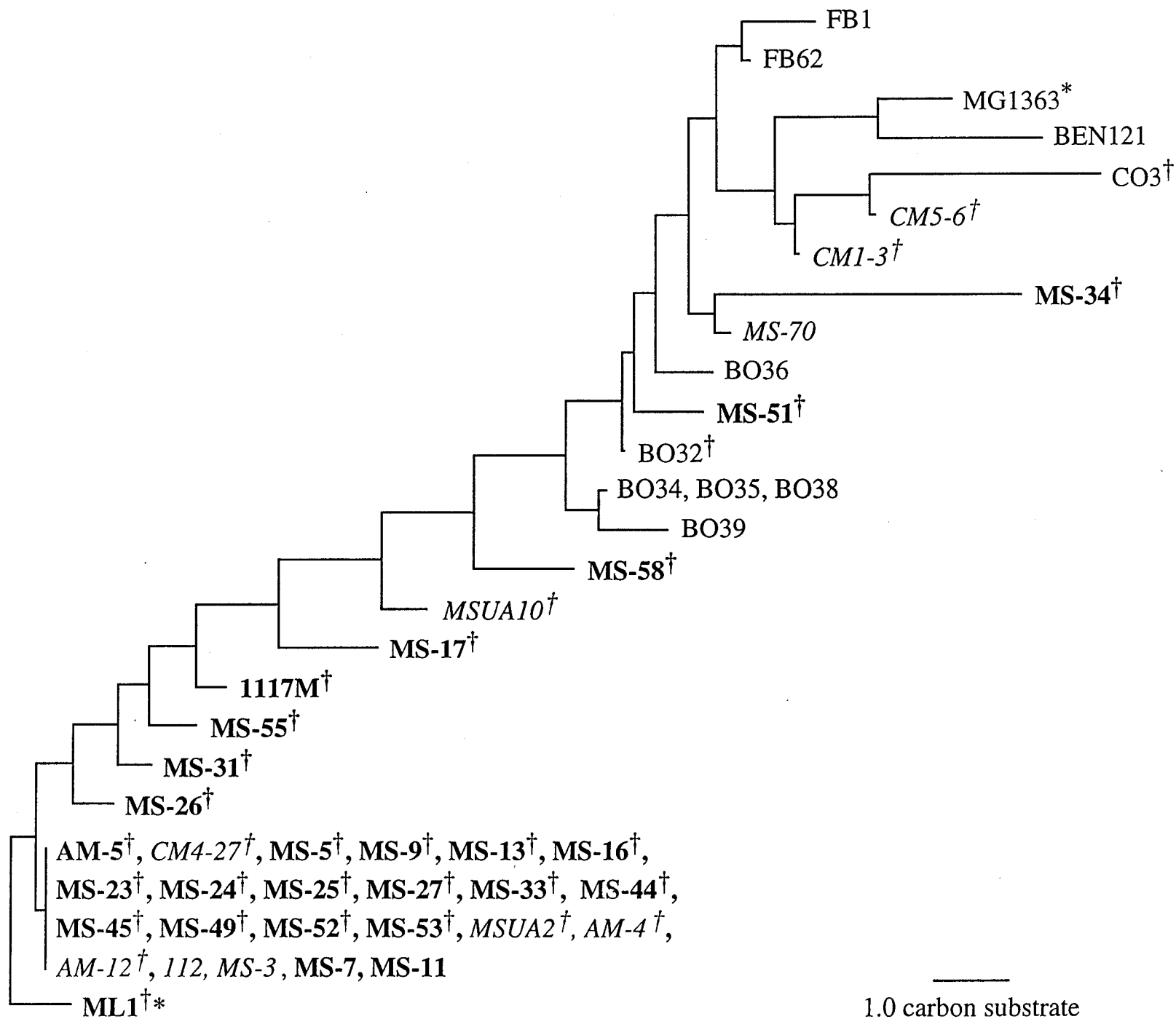
10

11 **Figure 2.** A dendrogram constructed by the neighbor-joining method from carbon
12 substrate utilization data shown in Table 3. Strains able to use the fewest carbon sources
13 appear at the left, with catabolic diversity increasing towards the right. Relationships
14 shown here correlated neither with relationships among *ldh* genes nor with phenotypic
15 subspecies designations. **Bold text:** phenotypically subsp. *cremoris*. Plain text:
16 phenotypically subsp. *lactis*. *Italic text:* phenotypic subsp. indeterminate. Asterisks (*)
17 indicate laboratory strains derived from commercial starter cultures. Daggers (†) indicate
18 strains belonging to the subsp. *cremoris* phylogenetic group in Figure 1.

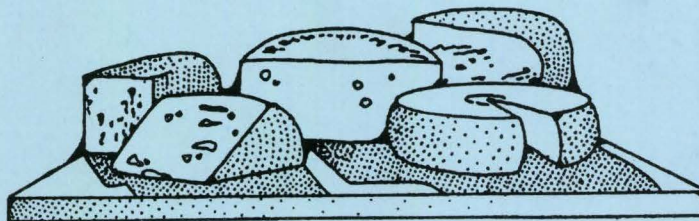
19



0.01 substitutions
per sequence position



**PHAGE CONTROL IN LACTIC
ACID BACTERIA: SUMMARY OF
AVAILABLE TECHNIQUES**



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PHAGE CONTROL IN LACTIC ACID BACTERIA: SUMMARY OF AVAILABLE TECHNIQUES

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Lactococcus lactis is an important industrial bacterium used to ferment milk to make cheddar and other types of cheeses. The role of *L. lactis* in milk manufacturing is to produce lactic acid, which helps curdle the milk, and produce metabolic products, which impart flavor to the cheese.

Bacteriophages (phages) are viruses that infect and kill bacteria. They co-evolve with the bacteria, and occur wherever bacteria are grown. Bacteriophages that infect *L. lactis* are found in raw and pasteurized milk, whey, and industrial equipment used to make cheese. Bacteriophages pose a constant threat of failed fermentation, which can result in inconsistent cheese quality, loss of milk, and wasted production time.

Historically, the bacteriophage problem was first recognized in the 1930's (Whitehead and Cox, 1936). Phage-resistant strains were isolated, but it was quickly realized that the immunity to the effects of the phage were short-lived, and not a solution to the problem. Phage infection was initially controlled with a strategy of rotating different mixed-strain cultures. Conceptually, the phage problem would be avoided by substituting a different mixture of strains before a contaminating phage could become a problem. Presumably, each mixture would not be susceptible to attack by the same phages. Unfortunately, the strains in each mixture were uncharacterized, and this led to cheeses of inconsistent quality. As the number of mixtures in rotation schemes increased, the risk of phage infections increased as well, because the increased number of strains just adds to the probability that lysogenic or mutant phages will find a compatible host (Lawrence, 1978).

Until the 1970's, the undefined mixture rotation was still the norm. Additional strategies to reduce the phage problem focused on trying to minimize the contact between the starter cultures and the contaminating phage. Thus, more emphasis was placed on the sanitation of equipment, closed vats to avoid air-borne phage, and aseptic propagation of the starter cultures instead of passaging from a single mother culture. Phage inhibitory media were developed, which reduce phage infections by chelating the Ca^{+2} required for the adsorption of phage to the surface of the starter bacteria.

In the early 1970's, the strains in the starter cultures were isolated and characterized, first in New Zealand and Australia, and later in the United States, Scotland, and Ireland (Huggins 1984; Klaenhammer, 1984; Lawrence *et al.*, 1978; Thunell and Sandine, 1981). The characterization included important metabolic traits, such as lactic acid production, milk protein breakdown, citrate utilization, and diacetyl, CO_2 , and acetaldehyde production, and the strains' sensitivities to various phages. The characterized strains became known as "defined" strains. With the understanding of each strain's characteristics, the defined strains could be combined in mixtures that produced consistent fermentations and cheeses of consistently high quality. The phage-sensitivity and -resistance profile of each strain made it possible to include from two to five phage-unrelated strains in each mixture. Importantly, because the composition of strains in the mixture was known, the whey from each batch of cheese could be tested on each of the strains separately. The rotation strategy with defined strains became one of substituting any one of the strains in the mixture that showed signs of phage in the whey, with another defined strain that would not propagate the contaminating phage. Ideally, each of the defined strains could be replaced with another strain with identical metabolic characteristics, but unrelated in terms of the phages that could infect. In practice, the defined strain rotation was an improvement over the undefined mixture rotation, and is currently used worldwide in nearly all large, commercial cheddar cheese production facilities (Thunell and Sandine, 1985). Phage infections occur a lower frequency in facilities that use the

defined strain rotation system Thunell *et al.*, 1981). As a consequence of the success of the defined strain concept, a constant demand for more and different phage-resistant strains has been created.

In the past, the approach to isolating phage-resistant strains of *L. lactis* has been to infect a pure culture of a defined strain with a phage, and isolate the bacteria that are able to grow in the presence of phage (Whitehead and Cox, 1936; Limsowtin and Terzaghi, 1976; Marshall and Berridge, 1976). The techniques used for this strategy are fast, and isolating phage-resistant strains is nearly assured. This strategy selects bacterial cells that have genetic alterations, usually affecting the receptor for phage on either the cell wall or plasma membrane. Unfortunately, selecting for phage-resistance in this manner is sometimes associated with negative physiological changes important for cheese fermentations, most notably the loss of fast lactic acid production (Marshall and Berridge, 1976; Jarvis, 1981).

Because of the research to learn more about the basic biology of *L. lactis* and its bacteriophages, a more directed approach to constructing phage-resistant strains has become possible. The directed approach has developed a new generation of phage-resistant strategies that improve upon the resistance, and/or reduce the negative side effects of strains selected with traditional procedures.

A directed approach must target for use at least one of the known, naturally occurring mechanisms of phage-resistance in *L. lactis*, which includes restriction and modification systems (Sanders and Klaenhammer, 1981; Froseth *et al.*, 1988), abortive infection (Klaenhammer and Sanozky, 1985; Laible *et al.*, 1987), and blockage of phage adsorption (Sanders and Klaenhammer, 1983; Sijtsma *et al.*, 1988 and 1990). In addition, mutations in the host DNA can prevent phage infection by altering the cell surface carbohydrates that act as phage receptors (Valyasevi, 1990 and 1994; Gopal and Crow, 1993), and plasma membrane components required for phage infection (Valyasevi, 1991).

Restriction/modification is a bacterial cell's mechanism for recognizing its own DNA, and distinguishing it from any invading DNA that may enter the cell. The bacteria modify its own DNA in a specific pattern, which differs from any invading DNA, including phage DNA. A restriction enzyme cuts into small pieces any DNA without the correct pattern of modification that enters the bacterial cell. When a phage attacks the bacterial cell, its DNA must enter the host. If the phage DNA is cut by the restriction enzyme, the phage can not propagate in the host, and the infection is cured.

Abortive infection is a bacterial trait that stops phage infection in its later stages, by interfering with the replication of the phage. Many genes have been associated with this trait, and each may have a different mechanism to produce the same outcome.

Blockage of phage adsorption is the result of an extra coating of cell wall carbohydrate that masks the primary phage receptor on the surface of the bacterial host. It is thought to physically block the initial attachment of the phage to the host cell surface, thereby precluding any possible infection.

Mutations in genes that control the structure of the cell wall can change the primary phage receptor on the surface of the bacterial host. This type of change decreases phage adsorption, and reduces the efficiency of infection.

Mutations in genes that make proteins required for attachment of phage to the membrane interfere with the entry of phage DNA into the host cell. If the phage DNA can not enter the host, it can not propagate. *pip* (see below) is such a gene in *L. lactis*.

Currently, two of the directed strategies for constructing phage-resistant strains are being used commercially, or are in the final stages of testing for commercial application: 1) The Klaenhammer single strain rotation strategy (Sing and Klaenhammer, 1993; Durmaz and Klaenhammer, 1995); and 2) The Geller receptor gene replacement strategy (Garbutt *et al.*, 1996; Geller and Garbutt, 1996).

The Klaenhammer strategy is to rotate variations of a single strain that differ only in phage defense mechanisms. The use of a single strain avoids inconsistencies in the strain's characteristics important for making high quality cheese. Each variation of the single strain contains a different restriction/modification and different abortive infection defense mechanism. In an optimized rotation schedule developed in the laboratory (Durmaz and Klaenhammer, 1995),

three variations of a single strain were rotated on a daily basis using the Heap-Lawrence starter activity test (Heap and Lawrence, 1976). At the start of each day, milk was inoculated with a different strain than the one used in the previous day. In addition, 10^5 phage per ml of each of two different phages, plus whey from the previous day was added. After 9 successive days, the strain showed no signs of failure, and actually reduced the level of added phage during each growth cycle. The unique and crucial aspects of this strategy are: 1) Each strain variation contains two defense mechanisms. Restriction/modification defense mechanisms are always leaky, and quickly fail by themselves. However, the back-up defense system (abortive infection) stops the few phage that always leak past the restriction/modification defense. Klaenhammer and colleagues have shown that the pairing of restriction/modification with abortive infection has an additive effect on the level of phage resistance. 2) If a mutant phage escapes the paired defense mechanisms of one strain variant, it is quickly adsorbed and destroyed by the next variant strain; 3) At least some of the defense mechanisms can be introduced into each variant strain by the technique of conjugal mating. The advantage to this is that the introduction of genetic traits into some commercial strains of *L. lactis* can be difficult using methods other than conjugal mating.

The Klaenhammer strategy shows that paired, naturally-occurring phage defense mechanisms can be an effective tool for constructing highly phage-resistant strains. The challenge for the dairy industry is to: 1) construct food-grade plasmids that encode the phage defense mechanisms for paired use, 2) identify naturally-occurring plasmids that each contain more than one phage defense mechanism, or 3) move multiple, naturally-occurring plasmids, each with a different phage defense mechanism, into single strains. Although plasmid-born defense mechanisms are attractive because of their mobility, they also pose technical risks, such as: 1) the instability of plasmids, 2) the possible requirement for high copy number to be effective, and 3) the incompatibility with other plasmids carrying important traits.

The second directed strategy was developed by Geller and colleagues (Geller *et al.*, 1993; Garbutt *et al.*, 1996; Geller and Garbutt, 1996), and is a product of basic research to elucidate the mechanism of the early steps in lactococcal phage infections (Valyasevi *et al.*, 1990, 1991, and 1994; Monteville *et al.*, 1994). The strategy is to delete the cellular component (receptor) from the surface of *L. lactis* that is required for attachment of the phage to the host. Without a receptor, the phage would be unable to identify the strain as a host, and no infection could occur.

Surface receptors were identified (Valyasevi *et al.*, 1990, 1991 and 1994), and the gene (named *pip* for phage infection protein) for one of the receptors was isolated and sequenced (Geller *et al.*, 1993). *pip* encodes a membrane protein of unknown cellular function. Most membrane proteins in *L. lactis* are transporters of nutrients or metabolic waste products, or structural components. The PIP protein has structural similarities to number of functionally diverse proteins, all of which are fibrous in nature. The common structural motif between PIP and such proteins as myosin, and the M proteins of *Streptococcus*, is called a helix-turn-helix repeat. The structural similarities do not suggest a functional similarity.

Mutations in *pip* cause a phage-resistant phenotype that appears absolutely resistant to a number of phages, all but one of which have a prolate headed morphology and are classified in the phage species c2 (Jarvis *et al.*, 1991). *pip* is required for prolate phage infection in at least two strains, and probably most strains that are attacked by prolate phages. *pip* is present in all strains of *L. lactis* that have been examined for *pip*, including subspecies *lactis* and *cremoris*, and biovar. *diacetylactis*, either by Southern hybridization (Babu *et al.*, 1995), or polymerase chain reaction (J. Krause and B. Geller, personal communication). When a strain with a mutated *pip* was challenged with a composite of phages in the Heap-Lawrence starter activity test under simulated cheese-making conditions, there was no phage infection of the *pip* mutant even after 10 successive days of growth. In fact, the strain with the *pip* mutation actually reduced the number of phages added to the milk, probably by adsorbing the phage to the cell wall. Under no condition has a mutant phage been found that could infect strains with a mutated *pip* gene.

In a directed strategy of phage-resistance, a technology has been developed to move a mutated copy of *pip* into different strains of *L. lactis* (Garbutt *et al.*, 1996). A nonsense mutation in *pip* was constructed *in vitro*, and exchanged for the normal *pip* on the chromosome of a

laboratory strain of *L. lactis* subsp. *lactis*. The *pip*-exchanged strain is resistant to the same phages as strains with spontaneous mutations in *pip*. The growth rate of the *pip*-exchanged strain in laboratory media, including M17G and a defined minimal medium, is the same as the wild-type strain. There is no evidence that the *pip*-exchanged strain differs in any way from its parental strain other than its phage-resistance.

It is important that the techniques used for constructing phage-resistant strains not alter the food-grade status of the strains. During one of the steps in the exchange of *pip* genes, antibiotic resistance genes must be temporarily introduced into the host. To make certain that all antibiotic resistance genes are removed after *pip*-exchange, four independent measurements were employed, including sensitivity to antibiotics, Southern hybridization, and two analyses with polymerase chain reaction technology. By all measurements, it was conclusively shown that the engineered strain does not contain antibiotic resistance genes or foreign DNA.

It is important for a number of reasons to show that the *pip*-exchange strategy can be applied to commercial strains. First, differences in strains often make it necessary modify technical methods. Second, unanticipated changes in the characteristics of *pip*-exchanged commercial strains may not become apparent until cheese is made from the engineered strain.

The commercial strain *L. lactis* subspecies *lactis* MM210 was subjected to the same *pip*-exchange techniques as the laboratory strain described above (Kraus *et al.*, 1996). The exchange of *pip* was confirmed, and the strain was tested for its growth rates and phage resistance. The growth rate of the engineered strain in laboratory medium (M17) was the same as the parental strain. Its ability to acidify and coagulate milk was unaltered. Two phages of the small isometric headed morphotype (phage species 335), and one of the large isometric headed morphotype (phage species 949) did not require *pip* for infection. No phages of the prolate headed morphology were available for this host. However, when grown without an intact cell wall, the prolate headed phage c2 was able to form plaques on strain MM210, but not on the *pip*-exchanged strain MM210ex. The evidence shows that *pip* can be exchanged in a commercial cheese strain with no apparent disruption of its ability to grow and coagulate milk. It also suggests that *pip* is not required for infection by phages of the phage species 335 and 949. The ability of phage c2 to infect strain MM210 but not MM210ex, demonstrates that the PIP protein is expressed in MM210, but not in strain MM210ex, and suggests that prolate headed phages require *pip* for infection of strain MM210.

The *pip*-exchanged commercial strain MM210ex is currently being tested further for its ability to acidify milk, and being prepared to make cheese.

The *pip*-exchange strategy has a number of important advantages over the traditional method of selected phage resistance or the paired plasmid-born, directed phage defense system: 1) Because *pip* is a chromosomal gene, and not a plasmid-born trait, stability is permanent, and loss of *pip* is not possible, as occurs with plasmid-born genes. Selection pressure for maintenance of *pip* is never necessary, as is common for plasmid-born genes. 2) *pip* replacement knocks-out, or deletes the PIP protein. Therefore high gene copy number is never a consideration for effectiveness, as it may be with plasmid-born genes. 3) Phages that can overcome their requirement for *pip* and infect *pip* mutants have never been found, despite rigorous attempts to find such evidence (Babu *et al.*, 1995).

Despite the advantages of the *pip*-exchange strategy, a number of important challenges remain: 1) The techniques used to introduce the mutated copy of *pip* into its new host may not be applicable to all strains. Some strains are inherently resistant to artificial transformation, and some native plasmids may be incompatible with the vector that carries the mutated *pip*. These technical hurdles may be surmountable, but more research would have to be done to address these issues. 2) *pip* is apparently not required for infection by the most prevalent species of phage currently found in the US cheese factories. It appears that *pip* mutants will not prevent phage infection by the type of phage that is currently most problematic. This situation could change, if selection for prolate headed phages were to increase. The number of prolate headed phages isolated in the past suggests that the dominant type of phage in industrial settings can fluctuate. It is encouraging that *pip* is effective against such a broad range of phages, which appear to include all the prolate headed phages, and perhaps some of the small isometric headed phages (phage species 936). Cloning of a

gene analogous to *pip*, but required for membrane attachment of small isometric headed phages of the phage species p335 is currently under investigation.

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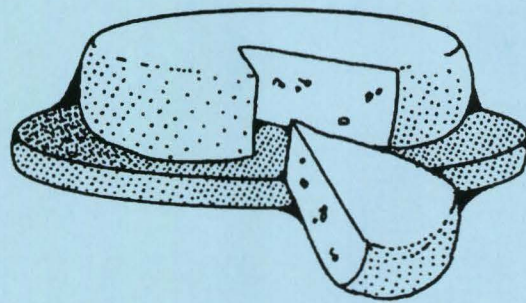
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**GENETIC MODIFICATIONS
OF CULTURES: WHERE DO WE
GO FROM HERE?**



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GENETIC MODIFICATIONS OF CULTURES: WHERE DO WE GO FROM HERE?

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Introduction

Because lactic acid bacteria (LAB) can be isolated from raw milk, it seems likely that fermented dairy products have been part of the human diet since milk was first collected in containers and held for a day or two. Over the centuries, these foods evolved into the distinct cheeses, yogurts, and fermented milks which are available today. It was not until the 20th century, however, that manufacturers of fermented dairy products recognized that substantial improvements in product consistency and quality were gained from the use of well characterized starter cultures. As knowledge of the physiology and genetics of dairy LAB expands, it seems even more certain that starter cultures with known, predictable, and stable characteristics will be the key to economic viability and innovation in tomorrow's dairy fermentation industry.

The development and refinement of biotechnology tools for LAB over the past 20 years now provides us with the capability to establish these qualities or even amend new traits in starter cultures. Increased quality, decreased production and storage losses, and expanded product diversity are some of the areas where biotechnology can contribute to economic growth in the dairy industry. With an estimated 800 industrial and academic laboratories worldwide now devoting resources to this area, it is clear that biotechnological approaches will have a significant role in the dairy industry. The 1980's and 90's were predominantly spent on the development of biotechnological techniques with applications in a few key areas such as bacteriophage resistance. The next decade should see a more concerted effort to utilize these techniques in a variety of dairy applications.

I. Biotechnology in Dairy Lactic Acid Bacteria

Modern biotechnology is rooted in the ability to genetically manipulate living cells in ways which heritably alter physiological properties of the organism. This technology is possible because of the discovery of various mechanisms which permit the introduction of exogenous DNA into the cell. Four of these processes have been discovered and developed in LAB: transduction, protoplast fusion, conjugation, and transformation.

Transduction

Transduction is a bacteriophage mediated form of gene transfer which involves the inadvertent packaging of host DNA within a phage particle during viral replication, followed by injection and expression of that DNA in a new bacterial host. Discovered in LAB by Bill Sandine

during the 1960s, transduction was the first form of gene transfer available for these microorganisms. A decade later, Larry McKay used transduction to show important milk fermentation properties in lactococci (i.e. lactose utilization and proteinase activity) were linked to plasmid DNA, and that integration of these genes into the chromosome dramatically increased the stability of these traits. These experiments revealed the importance of plasmid DNA in lactococci and demonstrated the first real avenue for genetic improvement of dairy starter cultures.

Protoplast fusion

Protoplasts are cells which have had their cell walls completely removed. With LAB, this is usually done through treatment with the enzymes lysozyme and/or mutanolysin. In the presence of polyethyleneglycol, protoplasts from different strains or species can fuse together and DNA may be exchanged. After fusion, the osmotically fragile cells are plated on media which facilitates regeneration of the cell walls, and recombinants which possess characteristics from each parent may be obtained by selection. Intra- and intergeneric transfer of plasmid and chromosomal genes among LAB has been demonstrated with this technique.

Conjugation

Conjugation is a natural form of gene transfer that requires physical contact between viable donor and recipient bacterial cells. A sequential model for the physical events in conjugal transfer has emerged from studies of fertility (F) plasmid transfer in *Escherichia coli*. In simplest terms, the steps may be divided into 4 parts; stable cell-cell pair formation, single-stranded DNA transfer from donor to recipient, complementary strand synthesis in the recipient, and dissociation of the mating pair.

Conjugation in LAB was discovered in the late 1970s and this gene transfer mechanism has since been widely used to study lactococcal plasmid biology and genetics. An important result from those investigations was the discovery that many industrially important traits in lactococci, including lactose and casein utilization, bacteriophage resistance, and bacteriocin production, are conjugative. This fortunate situation is of great practical significance to strain improvement efforts. Because conjugation occurs naturally, LAB which are genetically improved by this technique bypass many of the obstacles associated with the industrial application of strains which contain recombinant DNA. The utility of this feature was first demonstrated by Mary Ellen Sanders, who used conjugation to improve bacteriophage resistance in industrial strains of *Lactococcus lactis* and similar work has now been performed in several labs around the world.

Transformation

Transformation is the process wherein naked DNA molecules outside the cell are internalized and expressed. For LAB, the most effective transformation method developed to date is electroporation, a technique that uses a high voltage shock to create transient "pores" in the cell

membrane. Extraneous molecules, including DNA, are somehow able to pass through these pores and into the cell. During the past decade, electroporation has been used to reliably transform many (but not all) species of dairy LAB. This feature, combined with the commercial availability of reliable instruments, have made electroporation the method of choice for the introduction of exogenous DNA into LAB.

The development of transformation systems for LAB is of great significance because the ability to efficiently transform a particular cell is directly tied to the ease with which recombinant DNA (rDNA) technology may be applied to that organism. Use of rDNA technology also requires development of useful cloning vectors for gene delivery. Within the last decade, a number of sophisticated expression, integration, and protein secretion vectors for LAB have been developed. Though most are intended for laboratory research, several "food grade" vectors designed for application in foods have also been constructed.

II. Regulatory Aspects of Dairy Biotechnology

Substantial progress has now been made toward the isolation of genes important to dairy fermentations and the food grade delivery systems needed to introduce those genes into starter cultures. From this knowledge, several key applications for biotechnology in the dairy starter culture industry have been identified. Examples include:

- bacteriophage resistance*
- biogum production*
- flavor and texture enhancement; accelerated ripening of cheese*
- probiotics*
- production of bacteriocins and other natural antimicrobials*
- production of food grade enzymes and heterologous proteins*
- specialty markets: improved cultures for low-fat dairy products, decreased browning of Mozzarella cheese, etc.*
- stabilization of plasmid-linked activities*

Before many of these applications can be pursued, however, regulatory issues surrounding use of genetically modified cultures in human food must be addressed. In 1986, the U.S. Food and Drug Administration (FDA) issued a policy statement for regulation of biotechnology products in 1986, but important questions remain with regard to use of genetically improved starter cultures. How, for example, might FDA view a starter which contained a rDNA molecule derived entirely of DNA from the starter's own species? What about transformation of a native plasmid isolated from one food-grade bacterium into the same species or even another food-grade species? The second question is important because many important traits for milk fermentations are encoded by plasmid

DNA. If some of these plasmids were transformed into industrial strains, they would likely contribute an immediate refinement to the fermentation.

At present, FDA policy toward biotechnology is focused upon a case by case basis for review and approval. Biotechnology is an expensive process, however, and questions regarding the development and application of genetically modified starter cultures must be clarified if the industry is to embrace this science. Some insight into FDA's position is available from its 1992 policy statement on plants foods derived from biotechnology, since safety evaluation of genetically modified starter cultures would likely follow similar criteria (Jim Maryanski, 1996 pers. comm.). In that document, FDA states that "the key factors in reviewing safety concerns should be the characteristics of the food product, rather than the fact that the new methods are used." With this objective, FDA developed a series of flow charts which, in effect, address the genetic origins of new food varieties and the analytical profile of the food in comparison with its traditional counterpart.

FDA's guidelines for plant biotechnology are similar in principle to those published in 1990 by the International Food Biotechnology Council (IFBC). The recommendations put forward by IFBC, an expert group of food scientists and biotechnologists, were specifically designed to assist regulatory evaluation and safety determination of food biotechnology products. The decision tree developed by IFBC for safety determinations of whole foods, including those produced from genetically modified microorganisms, is shown in Table 1.

As we consider the safety of genetically modified starter cultures, it is important to realize that many species of dairy lactic acid bacteria were used as starter cultures before 1958, and thus may be defined as GRAS (generally regarded as safe) because of our experience based on common use in food (Jim Maryanski, 1996 pers. comm.). From this position, it is relatively easy to envision a number of genetic improvements to starter cultures that, based on the criteria put forward by FDA and IFBC, would likely have no impact on food safety or composition. IFBC anticipated that many innovations for biotechnology in food would fall into this category, and concluded that it would be an enormous drain on FDA resources to require GRAS (generally regarded as safe) affirmation petitions from every one. As a solution, IFBC suggested manufacturers utilize GRAS provisions which allow them to make an independent (i.e. in house) GRAS determination. They also recommended that FDA establish an informal procedure that would allow companies to inform the Agency of these determinations. It is my understanding that individuals and companies are now using these recommendations to address questions related to the development and application of genetically modified starter cultures.

Table 1. Decision tree for the safety evaluation of whole foods and other complex mixtures (from IFBC 1990).

Describe the product and characterize it in light of its genetic origins, then proceed to answer the following series of questions^a. Note Words in *italic* are defined.

Question	If:		Comments
	Yes/go to	No/go to	
1. Was the product developed only from genetic material derived from plants or microorganisms that are <i>traditional foods</i> or related nonfood species previously used as sources of genetic variation in developing and improving foods by traditional methods of genetic modification?	2	7 ^b	For a fuller discussion of acceptable genetic elements see Table 22. <i>Traditional foods</i> are defined in the Glossary.
2. Are the constituents in the food product only <i>inherent constituents</i> ?	3	4	<i>Inherent constituents</i> is defined in the Glossary.
3. Do these constituents (question 2) occur within the documented range for the parental traditional food?	5	5	Criteria for acceptable ranges of inherent constituents are presented in Chapter 6, Section 3.1.
4. Does the intake of <i>new constituent(s)</i> under the intended or reasonably expected conditions of use present <i>no safety concerns</i> ?	6	10	The terms <i>new constituent</i> and <i>no safety concern</i> are defined in the Glossary.
5. Can the intended or reasonably expected conditions of use result only in a pattern of intakes of individual constituents that does <i>not alter significantly present intakes</i> ?	6	Safety evaluation of constituents; go on to 6 or reject	The term <i>not alter significantly present intake</i> is defined in the Glossary. Safety evaluation refers to existing practices to ensure that a food product or constituent presents <i>no safety concern</i> .
6. Are the <i>significant nutrients</i> in the product within the expected range for the closely comparable <i>traditional foods</i> which the new food will replace?	Accept	Evaluate consequences and accept or reject	The term <i>significant nutrients</i> is defined in the Glossary.

Table 1 -continued

Question	If:		Comments
	Yes/go to	No/go to	
7. Is available knowledge and documentation adequate to characterize the <i>introduced genetic material</i> in terms of its origin and expected expression products and to ensure its acceptability for use in food? (Table 22)	2 and 4	8	Introduced genetic material means any incorporated DNA. Documentation should be adequate to support its inclusion in Table 22.
8. Are the expression products of the <i>introduced genetic material inherent constituents of foods</i> ?	9	Safety evaluation of new constituents; go to 4 or 10 or reject.	<i>Foods</i> in this context means any food, not necessarily the traditional counterpart food.
9. Are the expression products of the <i>introduced genetic material</i> present at concentrations inherently found in foods?	2 and 4	Safety evaluation of new constituents; go to 4 or 10 or reject.	<i>Foods</i> in this context means any food, not necessarily the traditional counterpart food.
10. Can the new constituents be removed, reduced to acceptable levels, or inactivated by processing?	2	Safety evaluation of new constituents and/or whole foods.	Food processing may be used to reduce or remove undesirable constituents.

*Procedures for product characterization are discussed on pages S138-140. In essence this consists of a description of the genetic origins of the food and an analytical profile of the food in comparison with its traditional counterpart.

^bIf the material is a new macroingredient such as single-cell protein, safety evaluation would be required along with the development of process and product specifications.

III. So Where Do We Go From Here?

With an avenue to address regulatory questions and considerations in place, steps must now be taken to expand the application of biotechnology in the dairy starter industry. Efforts to genetically improve dairy starter cultures should, in my opinion, follow an incremental path whose final goal is the judicious application of rDNA technology. Every step in this process will establish a precedent that should make it easier for related applications to follow. By building on each success, I believe we will also promote trust and respect between FDA and the dairy industry. Genetic improvements to starter bacteria which are already in place and which might be used as steps in the ladder leading to rDNA technology include:

Intraspecific exchange of native plasmid DNA

The destructive influence and cost of bacteriophage upon the dairy fermentation industry has been unparalleled among other fermentation industries. As a consequence, bacteriophage resistance in LAB has persisted as a central theme for genetics studies in these organisms. Genetics studies of lactococci have provided a large deposit of information on several mechanisms for bacteriophage resistance and the loci which encode them. As was mentioned earlier in this presentation, conjugation of phage resistance plasmids from *L. lactis* laboratory strains to commercial cheese starters has been used to enhance phage resistance in Cheddar cheese starters since the late 1980s.

Using conjugation and transformation of native *L. lactis* phage defense plasmids, Todd Klaenhammer's group at North Carolina State developed an innovative strategy which rotates different phage defense mechanisms within a single-strain starter system. In laboratory experiments, this system not thwarted bacteriophage proliferation, it actually removed contaminating phage from the medium.

Intergeneric exchange of native plasmid DNA

Increased production of Mozzarella cheese in the U.S. during the past decade has led to an emergence of *S. thermophilus* bacteriophage. Research in my lab has shown that transformation of *Pediococcus pentosaceus* and *P. acidilactici* with a native *L. lactis* lactose plasmid gives Lac⁺ pediococci which have good potential as replacement cultures for *S. thermophilus*. Phage susceptibility testing showed Lac⁺ pediococci were not sensitive to any viruses in whey samples collected over a one-month period from North American Mozzarella and Cheddar cheese plants.

Gene replacement

Gene replacement is a process that uses rDNA technology to inactivate one or more host genes. Unlike other rDNA applications which seek to introduce genetic material, gene replacement is used to remove part of the host's DNA so that one or more proteins are longer produced. Since foreign (vector) DNA is not left behind, strains which are engineered by gene replacement are genetically nothing more than a deletion mutant of the wild-type parental strain.

The utility of gene replacement for genetic improvement of starter cultures can be illustrated by the work of Dr. Bruce Geller at Oregon State University. Dr. Geller identified a lactococcal protein (Pip) that is required by many bacteriophages for DNA injection. Studies with spontaneous Pip⁻ mutants showed these strains were completely resistant to several phages and that loss of Pip did not affect lactococcal milk fermentation properties. Unfortunately, spontaneous mutants are capable of reversion, so gene replacement was used to construct strains with a permanent *pip*⁻ genotype.

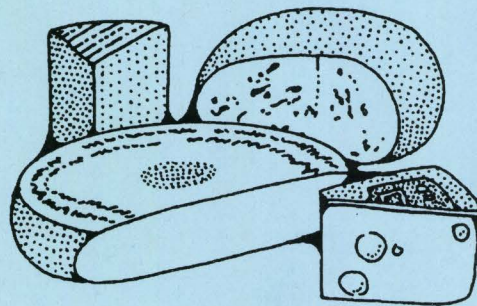
Summary

With the arrival of genetic tools for dairy LAB 20 years ago, the dairy fermentation industry entered a revolution which has now provided investigators with unprecedented power to assure the success of milk fermentations. Within this short period, important biochemical pathways have been elucidated, gene transfer and delivery systems were discovered and refined, gene expression and secretion signals were identified, and a large number of important genes were located, isolated, and examined at the DNA sequence level. With an improved view of regulatory considerations in place, the stage is now set for more widespread use of gene technology in the dairy fermentation industry.

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**MANAGEMENT OF CULTURES: THE
AUSTRALIAN EXPERIENCE**



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MANAGEMENT OF CULTURES: THE AUSTRALIAN EXPERIENCE

Gaetan K.Y. Limsowtin

Australia along with its close neighbour New Zealand has a great affinity with the United States Cheese industry, especially in the dairy starter field.

The 1970's saw the development of the defined starter system to cope with the increasing milk throughput and plant size and researchers from both sides of the Pacific have collaborated with each other over these last two decades. This tradition continues to this day.

During the mid 70's New Zealand opted for a single paired starter system that can be used on a daily basis for all plants. This system is still being used today with a triplet set of starter strains. By contrast Australia's starter system was more tentative mainly because of its dairy industry structure. The actual adoption of the defined starter system took a different direction in Australia. Unlike New Zealand where the New Zealand Dairy Board is the sole seller, each Australian company competes with each other for the local cheese market. Under these conditions, starter strain development was carried out by the laboratories of each individual plant even within the same company. Initially the phage derivation system worked adequately enough to enable individual plants to cope with the phage problem. But gradually as more experienced personnel left their positions or moved to other industries this plant-based strain derivation system became cumbersome and erratic in performance resulting in many failures. At the time, the dairy research laboratory of the CSIRO who had previously pioneered the factory derivation of new phage resistant strains was unfortunately curtailing its research program on starters. The cheese industry saw the need for a highly focused organization to research on starters and the major companies decided to fund their own Research and Development company resulting in the formation of the Australian Starter Culture Research Centre (ASCRC) in February 1992.

This talk will dwell on this new organization and its impact on the starter management scene in Australia since 1992.

What was the state of the starter research and supply in 1992?

At the time ASCRC was being set up, R&D activities in starter technology were quite low except for some significant basic work undertaken by Drs Barrie Davidson (University of Melbourne) and Alan Hillier (CSIRO) (Table 1). Each factory was deriving its own phage resistant starter strains achieving only partial success with large variations in performance still occurring. The main drawback of the system was that the flavour potential of the new strains and their taxonomic status were not investigated.

Phage problems were most prominent, causing very high levels of losses in product grade and manufacturing efficiencies. The participation of all main European commercial starter suppliers complicated the situation further.

It was amidst this difficult situation that the Australian cheese industry looked closely at its neighbour, New Zealand, and asked itself whether it could adapt such a system to suit Australian conditions (Table 2). The Dairy Research & Development Corporation, a primary producer corporation, was the catalyst in setting up the ASCRC. Its charter was simple but I have a huge continent to service (Table 3).

I was given a small laboratory within the old Dairy Research laboratories of the CSIRO at Highett in Melbourne. Within one year in the refurbished laboratory we were preparing to move to another larger facility situated on the western fringe of Melbourne where a large Food Research complex was being assembled.

My strategic approach in setting up this new starter research centre was to ensure that the industry gets what it wants ultimately - i.e. reliable and appropriate cultures for all their portfolios of cheese varieties.

On the way to achieving this goal an appropriate Research & Development program was set up and at the same time a commercial frozen starter production unit was constructed and commissioned within 18 months of ASCRC's launch.

Today I'm glad to report that ASCRC is functioning beyond expectation, its subscribers and the cheese industry have been well served by its achievements (Table 4).

Now I would like to elaborate further on starter management itself. How can we achieve proper starter management in the pluralistic context of Australia, contrasting sharply with the monolithic situation in New Zealand where starters are supplied solely from the New Zealand Dairy Research Institute?

It was evident the starter problems of Australia could not be resolved by a direct copy of the well insulated New Zealand system.

For Cheddar manufacture the majority of plants still used a bulk starter preparation step. Direct set starters were too expensive (> \$100/tonne of cheese) an option to consider. The use of a large number of strains was prevalent, and this situation was not likely to be changed overnight. Therefore a robust starter technology system needed to be devised.

We conceived a system built around a more reliable bulk starter fermenter. If one can be assured of a phage-free bulk starter preparation in a plant then virtually every starter strain can be used in a phage-unrelated rotation. Modern cheese equipment can preclude or at least limit phage contamination of raw milk to a workable minimum.

Our system makes use of new technology and a modern approach to characterizing strains to achieve a consistent production as well as a good flavoured cheese.

This leads me to the five elements that constitute the Australian Starter Management System (Table 5).

1) A UHT - based Bulk Starter Preparation Fermenter (Figure 1)

This new concept was developed by one of ASCRC's subscribers in collaboration with ASCRC and an equipment supplier. Basically it consists of a UHT plant attached to one or several bulk starter fermenters. Bulk starter media used in Australia are mainly skim or whole milk. The milk is

sterilized in the UHT plant and is then diverted to a chemically sterilized fermenter (presently 7000 - 10000 litres capacities). Chemical sterilization is achieved with an acetic acid / peroxide sanitizer after a normal CIP with sodium hydroxide at ~ 60°C .

Filtered air is fed continuously to the fermenter and is designed to prevent entry of bacterial and bacteriophage contamination. The bulk starter preparation room also receives HEPA filtered air in one plant. The fermenter is fitted with pH electrodes to control pH during growth by addition of alkali. After growth the bulk starter is cooled rapidly using a jacketed cooling system and then fed directly to the cheese vats (Table 6). All plants have designed their system without any holding starter vats - i.e. starters are fed from one fermenter while another fermenter is being set up. The large volume of each fermenter means that a starter preparation can be used over at least 2 days.

This new system is now operating in six large cheese plants with great success and another one will be built next year. The combined production of all these new fermenters will be > 80% of all the Cheddar production in Australia.

2) Culture Collection (Table 7)

A comprehensive starter culture collection (> 1000 strains) drawn from the private collection of each participating company, the CSIRO, ADCA (now defunct) and other dairy laboratories in Europe and USA has been assembled. This collection provides the wide base of genetic types from which new strains can be isolated or original ones simply re-used where appropriate. Characterization of strains is focused on the product requirements - activity in milk, sensitivity to salt and cooking temperatures, response to rapid cooling after cheese manufacture, phage/host relationships and flavour potential profile. Characterizing the flavour profile of the starters is given the same weighting as their phage sensitivities. This means that a strain that

consistently produces good flavoured cheese in a particular plant can still be used, provided the usual precautions are taken.

3) National Phage Monitoring System (Table 8)

This monitoring system is based on the routine analysis of cheese wheys for phages. Presence and levels of phage are reported back to the plant. The ASCRC laboratory examines the host range and the degree of virulence of the phage and the results are discussed frequently to ascertain the safety of a particular set of strains. The host range data are fed to a database that updates the record for each sensitive strain concerned. This system helps to build a phage/host relationship table and thus enables us to provide a safe starter rotation as well as back-up strains to each plant (Table 9).

4) Derivation of Phage-Insensitive variants (Table 10)

The direct challenge of starter strains with cell-free wheys and/or purified phage preparations is the main avenue of obtaining phage insensitive variants of a desirable strain. The degree of success with this method is low as most variants tend to be less active in milk (Figure 2).

We have been very careful not to use recombinant DNA means of achieving phage resistance. However the conjugal transfer of plasmids known to encode phage resistance genes appears to be safe and is within the limits of the Australian Genetic Modification Guidelines.

Thus we have transferred the well described plasmid pNP40 which encodes nisin resistance to other recipient strains. One of these resistant strains has been successfully used commercially. We are presently examining other strains that are still insensitive to phage in Australia with the view of transferring any putative phage-resistance encoding plasmids.

5) Central Supply of Frozen Bulk Starter Inoculum (Table 11)

A production unit capable of supplying the whole Australian Dairy Industry with frozen bulk set inoculum has been trialled since 1993 and become commercially operational in 1995. Starters grown in pH-controlled fermenters are frozen in 150ml pottles and despatched to plants at intervals. This system can deliver starters to all plants within a very low price structure (A\$1 - \$2/tonne cheese). Both mesophilic and thermophilic starters are currently being supplied to the plants.

Performance of the Starter Management System

Over the last three years the starters supplied to each plant have performed consistently well. The number of strains used by member companies of ASCRC has decreased steadily from ~ 60 in 1992 to ~ 46 strains during the 1995/96 season. The actual number of strains (mesophilic and thermophilic) used to manufacture 80% of the companies' production was only 29 (Table 12).

One strain (ASCC47) is virtually used in all factories and remained insensitive to phages for three years until this year.

Among the strains used at least five strains have been used previously during the 1970's and we have shown that they can be used again by the industry. Phages have eventually appeared for all of them but provided the phage host ranges are well characterized the strains concerned can be used safely in paired rotations.

Cheese flavours have improved because of the judicious strain selection based the flavour potential as well as absence of bitterness. A significant proportion of Australian cheese export goes to the Asian countries where bitterness is an important defect. The use of PepN peptidase activity as a guide to select the best pairing combination has worked extremely well.

In this way we have been able to learn from commercial experience that strains with low PepN activities should be avoided. This empirical approach has served us well in reducing the general level of bitterness in Australian cheeses.

The frozen starter production unit has provided a very efficient service to the industry by responding to all their needs. Starters can be despatched to Queensland (3,000 km from Melbourne) by road transport within 4 days on dry-ice as a refrigerated cargo. The unit is capable of supplying all Australia's needs based on the present bulk starter preparation system i.e. using < 1000 pottles per season for a plant producing 25,000 tonnes of cheese.

Technology Transfer Activities

As part of the overall starter management scheme, ASCRC is eager to supply a complete service package covering both starter supply and other microbiological aspects of the cheese-making process (Table 13).

I have taken two case studies to illustrate the close linkages that we maintain with the local industry through our technology transfer program.

Case I: Accumulation of 'thermo-resistant' bacteria (Table 14)

This case relates to the widespread adoption of UF for cheese milk standardizing. In Australia five factories manufacturing > 80,000 tonnes Cheddar/annum are currently using UF as part of their standardizing procedure. The problem area lies with the hot UF system where thermophilic organisms have accumulated in the pasteurized vat milk. In several cases the contaminating organism can reach high numbers in the final cheese ($\sim 1 \times 10^8$ cfu/g cheese). Invariably the contaminant has been a *Streptococcus thermophilus* strain. Normally this organism is not specially identified in a standard Cheddar plant laboratory monitoring program.

Standard tests for thermophilic (55°C) and thermoduric (growth at 30°C after pasteurization at 62.5°C/30 min) organisms are inadequate to identify *S.thermophilus*. ASCRC has successfully introduced a simple assay on M17 incubated at 42°C to identify *S.thermophilus*. This has been adopted in industry to monitor *S.thermophilus* contamination in pasteurized milk and cheese. When the

level of contamination is heavy ($> 1 \times 10^6$ cfu/ml pasteurized milk) the effect on cheesemaking becomes noticeable by the faster cheesemaking times after drying. The net effect is noted during cheese maturation when the ripening rate progresses faster than normal while development of bitterness is increased.

The growth of *S. thermophilus* and lactobacilli in UF plants with long continuous operating times can only be controlled by regular washing. This is costly to the processor and now we are seeing a shift to low temperature UF ($\sim 10^\circ\text{C}$). One plant has successfully used low temperature UF for three seasons and others are making similar changes. However, it will be necessary to monitor the possible rise of a psychrophilic and psychotrophic bacterial population to fill this new ecological niche.

Case II: General Starter Inhibition (Table 15)

The consistent performance of starters in any factory is largely dependent upon the cheese milk, assuming the rotated pairs or multiples of single strains have equivalent activities. Invariably at the beginning of a new dairy season the starter performance is erratic and new phage strains seem to appear during that time. In Australia we have observed a noticeable variation in the starter inoculum size for a given cheese make. In the absence of bacteriophage and veterinary clinical antibiotics this partial inhibition of starters has been explained in terms of lactoperoxidase system (LP) induction or other farm related chemical or antibacterial agents. In our investigations we have had three occurrences when the inhibition was due to growth of wild antimicrobial-producing lactococci. Levels of 10^5 cfu/ml in the raw milk were sufficient to cause significant slowing down (requiring 50% more starter). The first milk vats coming from one raw milk storage silo were lost due to complete inhibition of the starter.

The predominant cause of starter erratic performance is still unresolved because the problem does not last very long in a particular factory and thus escapes detailed investigation.

If one examines the level of starter inoculum used over a short period of time (Figure) it is easy to recognize that the starter is inhibited by an external factor which in this case can be relieved by heating at 85°C for 10 minutes. The LP System is prime suspect. In Australia the natural level of glycosides in the pasture is higher than the threshold level of 10mg/litre for LP induction while the enzyme lactoperoxidase is always present in abundance. Therefore in Australia the only limiting factor for the induction of the LP system is H₂O₂. As lactic acid bacteria do not have a catalase to break down the H₂O₂ formed during the initial stages of growth in milk containing high levels of dissolved oxygen, their growth is inhibited both by the H₂O₂ itself and by the induced LP system. Presently this problem is being addressed in two ways:- firstly, by checking for air leaks in all pipe reticulation and pumps; secondly by attempts to reduce the effects of dissolved oxygen in milk e.g. the use of CO₂. Carbon dioxide is currently used in Europe (France) to acidify milk before cheesemaking. The resulting lower pH gives rise to a firmer clot and less rennet is required which together increase overall cheese yield as well as producing less bitterness (due to rennet hydrolysis of casein) during maturation.

Future Outlook

ASCRC Research & Development activities will continue especially in developing better starters for a new wave of cheese types. Traditional varieties will still attract a significant proportion of our effort as more market segments are opened further.

Conclusion:

Our approach to starter management in Australia is still evolving. I am very keen to promote novel R&D techniques for fast adoption by the industry. For example we will soon trial an immuno-PCR rapid test for phage detection developed by one of our PhD students. How will a PCR fit in a dairy laboratory? Time will tell, but it illustrates our intent to move the dairy fermentation industry rapidly to the

cutting edge of scientific development with benefits accruing to the manufacturing companies and farms.

Starters in Australia 1992
R&D and Supply

- **R&D - Low level within CSIRO Dairy Research Laboratory**
- **Basic Starter Research - University of Melbourne /CSIRO**
- **CSIRO culture collection**
- **Factory derived culture collection**
- **Australian Dairy Culture Association collection**
- **Each factory deriving own phage resistant (~ 80% of all Cheddar Manufacture) variants**
- **Little external technical support for industry**
- **All main European commercial starter supplies were operating in Australia**

**RATIONALE FOR THE ESTABLISHMENT OF THE
AUSTRALIAN STARTER CULTURE
RESEARCH CENTRE**

- I) Starter cultures and their management are pre-competitive**

- ii) Australian Dairy Companies will optimize their individual expenditure on starters by pooling all their resources**

- iii) The companies will retain control on the Research & Development direction**

- iv) R&D benefits and outcome will flow directly and quickly to companies for implementation**

- v) Intellectual property resides collectively within the industry**

Charter for ASCRC

- **To Supply R&D in Starter Technology as directed by the Dairy Industry**
- **To provide industry with technical support (Cheese & Starter technology) through effective technology transfer**
- **To provide appropriate and performing cultures to the industry**
- **To assist in coordination of national R&D in lactic acid bacteria**

ASCRC Subscribers 1996

	<u>Cheese Production</u> <u>(Tonnes)</u>
The Bega Co-operative Society Ltd	~ 8,000
Bonlac Foods Limited	~ 45,000
Dairy Vale Foods Limited	~ 13,000
Dairy Farmers Co-op	~ 13,000
Murray Goulburn Co-operative Soc. Ltd	~ 53,000
Warrnambool Milk Products Ltd	~ 25,000
United Milk Tasmania	~ 18,000
Total: ASCRC Subscribers	<hr/> 175,000 tonnes
Total: Australian Cheese Production	216,000 tonnes

Elements of the Australian Starter

Management System

- ◆ **UHT-based Bulk Starter fermenter in cheese plants**

- ◆ **Comprehensive Starter Culture Strain bank**
 - **Product-oriented starter characterisation**
 - **Phage / Host Relationship table**

- ◆ **National Phage Monitoring System**

- ◆ **Derivation of phage-insensitive variants**

- ◆ **Centralised supply of frozen bulk starter Inoculum**

UHT-based Bulk Starter fermenter
for Cheese plants

- ◆ **Concept developed by the cheese industry and ASCRC**
- ◆ **All milk based media can be used**
- ◆ **UHT unit ensures media sterility**
- ◆ **Bulk starter unit sterilized chemically**
- ◆ **HEPA filtered air supplied to unit**
- ◆ **Water seal not necessary**
- ◆ **Efficient jacketed cooling provided
(37°C 8°C in < 1h)**
- ◆ **Large volume**
- ◆ **pH-control optional**

Starter Culture Collection

- **Flavour Potential**
 - Cheese Trials
 - Proteinase/Peptidase/Sulphur degrading enzymes
 - Cheese Slurries

- **Phage Sensitivities of strains**
 - Phage/Host Relationship (regionally based)
 - 'Old' performing strains

- **Other strain attributes**
 - Biochemistry of sugars
 - DNA characterisation
 - Temperature Sensitivities
 - Salt Sensitivities
 - Activity Test
 - Cheese Trials

National Phage Monitoring System

- * Weekly analysis of pooled wheys from each plant**

- * Prompt report on phage types and levels**

- * Purification of phages**

- * Host range determination**

Strain Usage System in Australia

	<u>Mesophile</u>	<u>Thermophile</u>
 <u>Without Rotation</u>		
2 - strains	√	√
3 - strains	√	
4 - strains		√
 <u>With Rotation</u>		
2 - strains x 2	√	
2 - strains x 3	√	
3 - strains x 2	√	
4 - strains	√	

Derivation of phage-insensitive variants

- **Direct challenge with cell-free wheys**

- **Direct challenge with purified phage preparation**

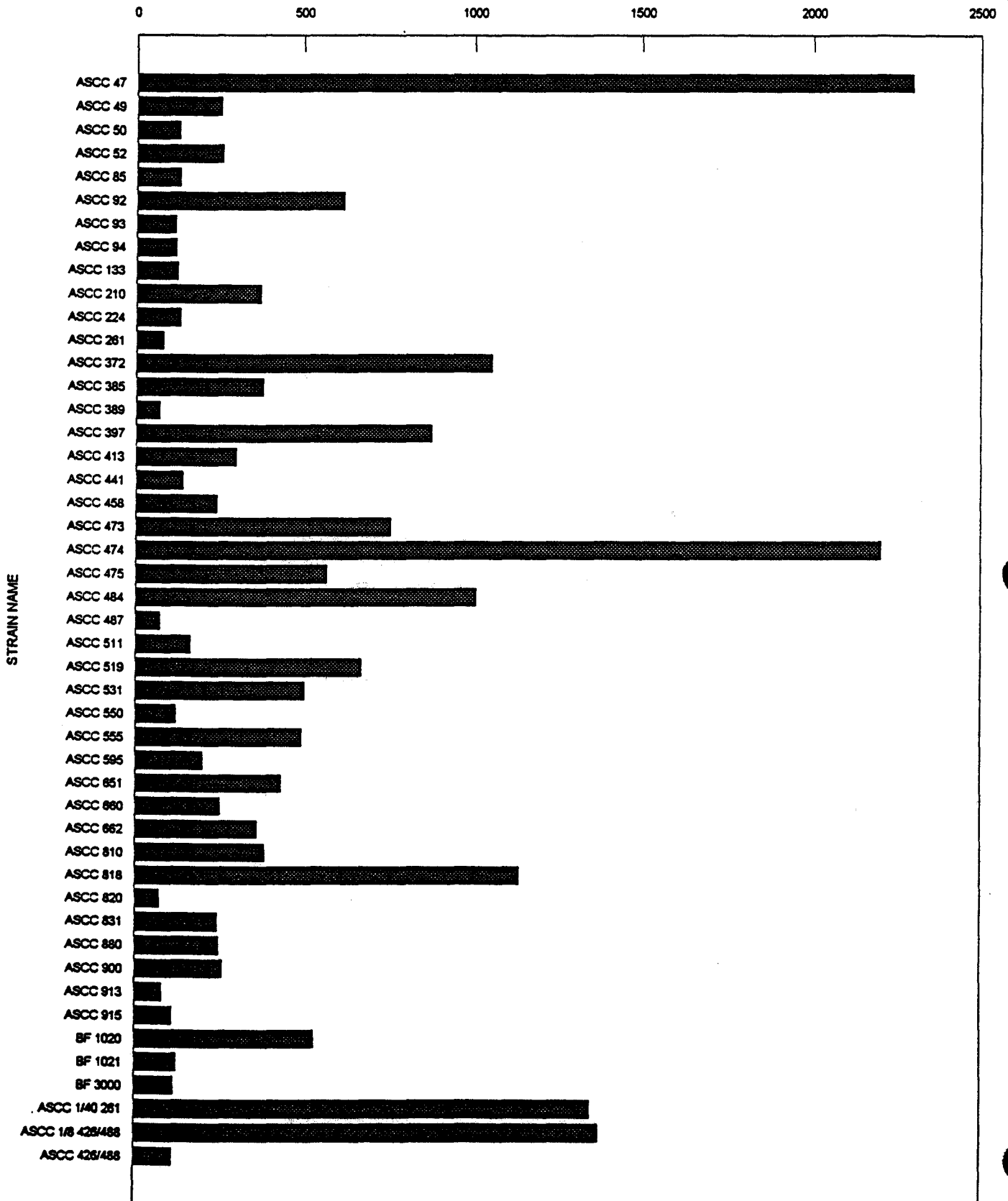
- **Natural transfer of phage-resistance encoded plasmid**

Centralized Frozen Bulk Set Inoculum Supply

- ◆ **ISO Certified production laboratory**
- ◆ **Mesophilic strain stock**
- ◆ **Thermophilic strain stock**
- ◆ **Frozen Inocula for Laboratory Activity test and phage monitoring**
- ◆ **Frozen inocula for milk starter activity**

POTILE PRODUCTION JUL 1995 - JUN 1996

NO OF POTTLES PRODUCED JUL95 - JUN 96



Active Areas of Technology Transfer

Table 13

- **Bulk Starter Preparation**
 - Neutraliser system
 - Air filtration
 - Heat treatment system
 - Individual requirement
 - Maintenance
 - Quality Control
 - Cooling regime
- **Starter Rotation**
 - Phage/Strain relationship
 - Manufacture/process
 - Flavour consideration
- **Cheese Flavour/Quality**
 - Grading sessions
 - Correlation with manufacture
- **New Products**
 - New Cheese varieties
 - Yoghurt
 - Whey ingredients
- **Milk Quality**
 - Establish causes
 - Remedial action
- **Non-starter Lactic Acid Bacteria**
 - Impact on cheese quality
 - New hygiene standard
 - Methodologies
- **UF Technology in Cheesemaking**
 - Cleaning regime
 - Process (starter impact)
- **Bulk Frozen Starter Trial**
 - Initiate & Monitor trials and progress
 - Training
- **Cheese Maturation Trial**
- **Monitoring of**
 - Residual Lactose
 - and Lactate isomers in cheese

Case Studies

I: UF - related

- Introduce UF to Cheesemaking
- Low concentration UF
 - Increase net vat capacity
 - Lower rennet usage

Implications:

- Hot UF - Thermophile
- Cold UF - Still under study
- Develop monitoring system for *S.thermophilus*
- Mid wash run-timing
- High Lactobacilli
- Ca-lactate monitoring New area of R&D

Case Studies

II General Starter Inhibition

- **Loss of first filled vats**
- **No phage detected**
- **No detectable antibiotics**

Causes of Inhibition

- **Induction of lactoperoxidase system
= (O₂ overloading)**
- **Growth of psychotrophic bacterioci
producing lactococci**

Solution

- **Ensure low milk temperature ex farm**
- **Ensure maintenance of low temperature**
- **Pre-processing testing**

Figure legends

Figure 1. Schema of a modern pH-controlled bulk starter unit in Australia.

Milk is sterilised in the UHT plant and fed at the appropriate temperature into a starter fermenter vessel presterilised chemically (Chlorine or H_2O_2 /acetic acid) by a cleaning-in-place system. Sterile air from a compressor is fed into the fermenter via a HEPA (High Efficiency Particulate Air) filter and is allowed to bubble out gently through the water seals around the stirrer shaft and inoculation port. After inoculation the pH electrode monitors a constant pH via a controller and an alkali dosing pump. The stirrer is kept on throughout the whole incubation period to ensure thorough mixing of the intermittent alkali additions and stirrer speed is deliberately kept low to prevent excessive air incorporation into the starter medium. A temperature probe also monitors the set temperature which is kept constant by means of hot or chilled flow into the water jacket of the fermenter. At the end of the fermentation, chilled water is used to cool the bulk starter to $4^{\circ}C$ which allows the starter activity to be maintained for up to 48 hours.

Figure 2. Methods for selection of phage-resistant starter strains.

A. The New Zealand system.

A tube of sterile milk is inoculated (1:100) with freshly-clotted culture of the starter strain (S), a sample of filtered (cell-free) factory whey (FW) and a mixture of purified factory phages (PP). The tube is incubated for 5 h through a Cheddar manufacture temperature profile. The pH is measured and compared with the pH of a control milk tube to which only the starter strain (no whey or phage) had been added. If the pH of the control tube is more than 0.2 pH units lower than the tube containing whey and phage, the strain is deemed to have failed and would not be considered suitable for factory use. If the strain passes this test (cycle 1 in Figure 2A) the test is

repeated (cycle 2), this time including whey from the first test (recycled whey, RW) in addition to factory whey and purified phages. The same pH criterion is applied to this test - a strain that fails is not used in factories. The tests are continued for a total of seven cycles. A strain that passes all tests is deemed to be sufficiently resistant to the phage population of the factories (*i.e.* phages in factory wheys) and to phages from the accumulated phage collection to be suitable for factory use. If a strain passes the early cycles but fails one of the later cycles, the test culture is allowed to clot at 22°C overnight and single-colony isolates are obtained. Several of these isolates are purified (three sequential single-colony isolations) and tested for desirable starter attributes including flavour, acid production and phage resistance. Isolates that pass all tests can be used to replace the phage-sensitive parent strain in production.

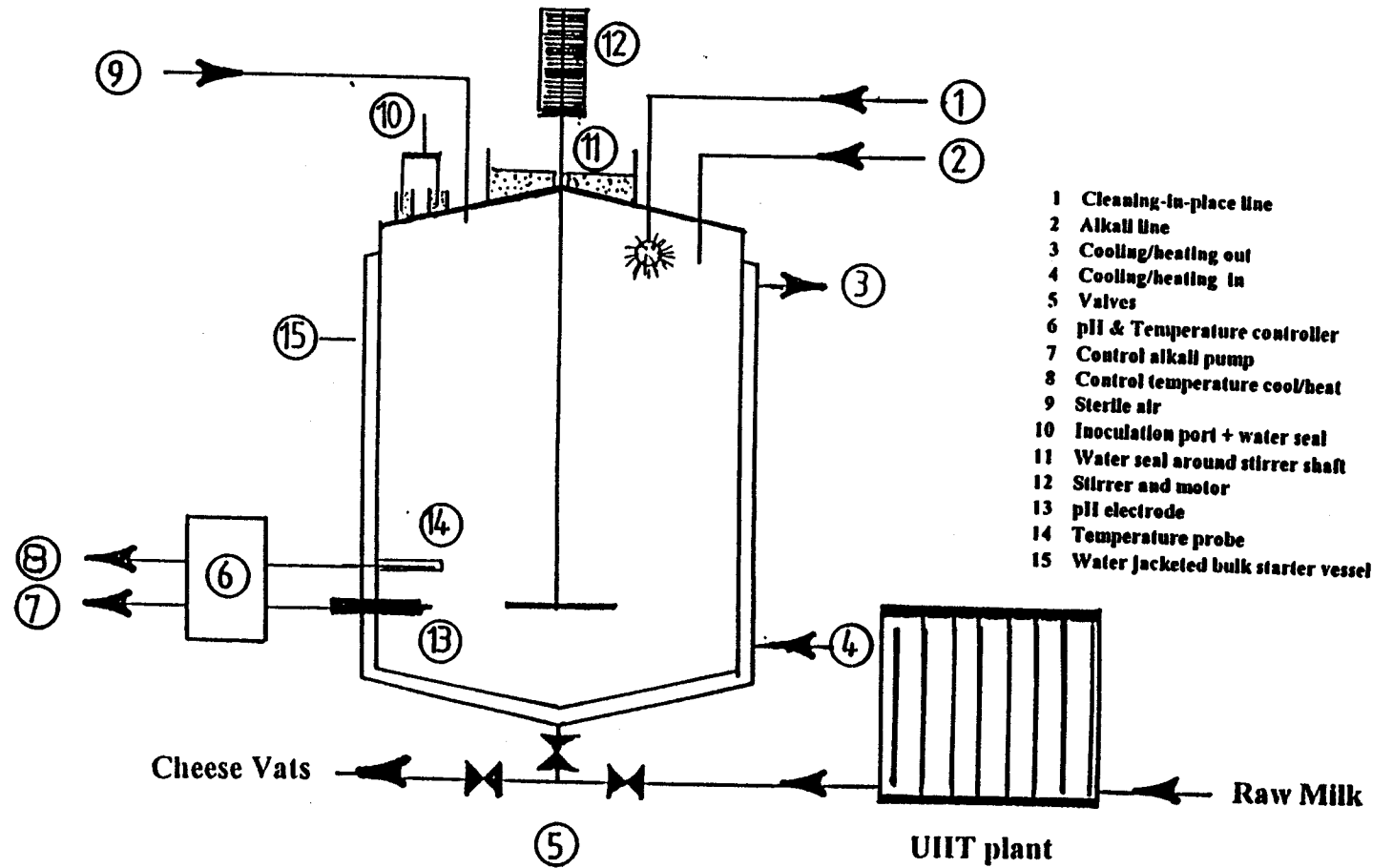
B. The Australian system.

This procedure was designed to be performed by trained factory staff rather than in a central laboratory. The presence of a disturbing phage in the factory is detected using an acid-production activity test in the presence of filtered factory whey. If a phage is detected, a large volume of sterile milk (0.5 - 1 litre) is inoculated (1:100) with a freshly-clotted milk culture of the starter strain (S) and filtered factory whey (FW). The culture is incubated through a Cheddar manufacture temperature profile and then at 25°C - 30°C until it coagulates (typically 12 - 36 hours). Single-colony isolates from this culture are tested for desirable starter attributes including flavour, acid production and phage resistance. Isolates that pass all tests are used to replace the phage-sensitive parent strain in production.

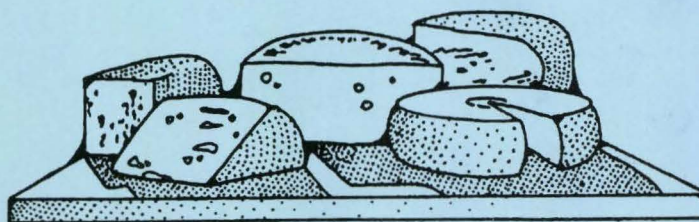
Figure from G.K.Y.Limsowtin, I.B.Powell and E.Parente (1995), *Types of starters in Dairy Starter Cultures*, T.M.Cogan and J.-P.Accolas (eds), VCH, New York.

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Figure 1



**PHYSIOLOGICAL STUDIES LEADING
TO THE CONTROL OF DEBITTERING
PROPERTIES OF LACTIC ACID
BACTERIA IN CHEESE**



**GERRIT SMIT
NETHERLANDS INSTITUTE FOR DAIRY RESEARCH**

PHYSIOLOGICAL STUDIES LEADING TO THE CONTROL OF DEBITTERING PROPERTIES OF LACTIC ACID BACTERIA IN CHEESE.

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SUMMARY

A laboratory assay was developed to measure the debittering capacity of lactic acid bacteria. A bitter-tasting peptide is incubated with bacteria in this assay and degradation of the peptide is monitored by reversed-phase HPLC. Several cultures were tested and large differences in activity were found among different strains. Using this assay, we were able to isolate strains with strong debittering activity. Interestingly, for a number of strains the growth conditions appear to play an important role in their debittering activity, with sensitivity for lysis as the most likely explanation for this phenomenon. Cheese experiments confirmed that the bitter assay can be used to predict bitter formation in cheese and show that bitterness in cheese can be prevented.

1 INTRODUCTION

Bitterness is one of the most common off-flavours in cheese (5, 11). We have focused on the possibilities to predict and control the bitter-degrading abilities of cheese (adjunct) cultures, which are used for accelerated ripening or flavour modification of Gouda cheese. The bitter-tasting C-terminal part of the β -casein, the so-called C-peptide (a.a. 193-209), formed by the action of rennet and starter organisms, is a major cause of bitterness in Gouda cheese (8, 11) as well as in Cheddar cheese (5). It has been found that the use of certain starter cultures and/or adjunct cultures give especially rise to the development of a bitter taste in cheese and therefore are characterized as 'bitter' or 'non-bitter' strains (11, 12). Based on this, an HPLC method was developed allowing the quick monitoring of the formation and degradation of the C-peptide. Several lactic acid bacteria strains have been tested for their ability to degrade this peptide in relation to the growth conditions of the strains. Moreover, cheese experiments were undertaken to confirm the results from the laboratory experiments.

2 MATERIAL AND METHODS

2.1 Bacterial strains, culture conditions and treatments

Strains were obtained from the NIZO culture collection. Strains were routinely stored at -135°C and grown in low-fat milk at 20°C (the Bos starter culture), 30°C (mesophilic lactococci), or 37°C (thermophilic strains). Cultures were cultivated in sterilized low-fat milk under acidifying or pH-controlled conditions. In the latter case the cultures were

maintained at a pH of 5.7 using 6.5 M NaOH as the neutralizing component. Lysed cell extracts were obtained by sonication of a stationary phase culture using a Heat Systems Sonicator XL for 4 times 30 sec at 0°C.

2.2 Purification of C-peptide and HPLC analyses

C-peptide was purified according to Vreeman *et al.* (13). Degradation of C-peptide upon incubation with bacterial cells was monitored by HPLC. Samples were prepurified (see below) and analyzed on a C₁₈-reverse phase column (PLPS 300 Å, 150 x 4.6 mm). A 10-90% acetonitrile gradient was used as the mobile phase in the presence of 0.1% TFA in both buffers. Eluting compounds were monitored at 260 nm.

2.3 Cheese trials and analyses

Gouda cheese was made from 200 L portions of pasteurized (10 s, 74°C) milk as described previously (9). Cheese milk was inoculated with 0.7-1.0% starter culture grown for 16 h in low-fat milk and, if appropriate, 2.5% adjunct culture was added. The adjunct cultures were cultivated for 40 h to minimize acidifying activity (see below). Cheeses were ripened for up to 6 months at 13°C and analyzed at various intervals. Organoleptic analyses were performed at 6, 13 and 26 weeks with a trained panel. Particular attention was focused on the bitterness of the cheeses, which was scored on a scale from 0 (no bitterness) up to 4 (extremely bitter).

2.4 Analysis of volatile compounds

Volatile components in cheese were identified using purge-and-trap thermal desorption cold-trap gas chromatography mass spectrometry (PTTDCT-GC/MS) as described previously (1, 6). Briefly, 20 ml of a cheese slurry, obtained by homogenization of a mixture of cheese and double-distilled water (1:2 w/w), was prepared and used immediately after preparation. The samples were purged with 150 ml/min helium gas for 30 min at 40°C and volatile components were trapped on an absorbent trap containing Carbotrap (80 mg, 20-40 mesh, Supelco and Carbosieve SIII (10 mg, 60-80 mesh, Supelco). The trapped compounds were transferred onto a capillary column of a gas chromatograph using the Chrompack PTI injector (Chrompack, Middelburg, The Netherlands) in the TDCT mode, by heating the trap for 10 min at 250°C. A narrow injection band was achieved by cryofocusing at -100°C. The conditions for the chromatographic separation and mass spectrometry have been described earlier (14). Structures were assigned by spectrum interpretation, comparison of the spectra with bibliographic data and comparison of retention times of reference compounds.

3 RESULTS AND DISCUSSION

3.1 Development of the bitter assay and screening of cultures

A laboratory assay was developed in which C-peptide was incubated with bacterial cultures. Three ml of the cultures was harvested at the stationary phase of growth, milk components were clarified by raising the pH to 6.8 (NaOH) and addition of sodium citrate to a final concentration of 1%, and centrifugated in an Eppendorf centrifuge (14,000 g for 15 sec). Subsequently, the cell pellets were resuspended in 50mM citrate, 500 mM NaCl buffer (pH 5.4) to an optical density ($A_{578\text{nm}}$) of 2.5, and incubated with 100 µg C-peptide at 30°C. With respect to the salt concentration and pH, the conditions in the assay resemble those in the cheese. At regular intervals 250 µl samples were taken. Enzyme activity in these samples was destroyed by heat treatment (20 min at 66°C) and, subsequently, the samples were clarified by centrifugation, and the amount of C-peptide

determined by HPLC. As shown in figure 1, it appeared that the amount of C-peptide decreased during the incubation.

Several lactic acid bacteria, grown under acidifying conditions in milk, were screened in the assay. For each strain tested, the debittering activity in the assay was expressed as the decrease in C-peptide per hour and per amount of cells (A_{578} units). The strains were found to differ significantly in their ability to degrade the bitter-tasting C-peptide (Table 1). Some strains were hardly able to degrade the C-peptide, while others were capable to degrade the peptide very fast. Moreover, the resulting peptide profiles which were formed during the degradation of the C-peptide were different for a number of strains.

3.2 Effect of growth conditions on debittering activity

Figure 2 shows that growth conditions of the culture significantly affect the debittering activity. In general, cells grown under pH-controlled conditions have a stronger debittering ability than cells grown under acidifying conditions. Therefore, strains cannot simply be marked as 'bitter' or 'non-bitter'(12). This result might open the possibility to use cultures for cheese-making which were previously disregarded because they were marked as 'bitter'.

3.3 Possible role of cell lysis in debittering activity

Since the C-peptide is too large to cross the cell membrane of the lactic acid bacteria and such peptides are not degraded by the cell envelope proteinase of the lactococci (3, 4), and Tan *et al.* (10) proposed lysis of starter cultures to be involved in debittering activity, we hypothesized that lysis of the bacteria might play an important role in the ability of the cells to degrade the C-peptide. To test whether the different cultures have the enzyme potential to degrade the C-peptide, lysed cell extracts were tested in the assay. All strains tested were found to be able to degrade the C-peptide upon lysis (Fig. 2), therefore, it can be concluded that differences in the release of intracellularly-located enzymes (e.g., peptidases such as PepN [10]) play a major role in debittering activity.

Preliminary results indicate that different growth conditions (see above) of the bacteria result in different sensitivity of the cells to lysis; e.g., cultures grown under pH-controlled conditions appear to be more sensitive to lysis than cells grown under acidifying conditions. This difference might explain the differences in debittering activity (Fig. 2). Moreover, it cannot be excluded that different levels of enzyme activity as described for some peptidases by Meijer *et al.* (6) may also contribute to this difference.

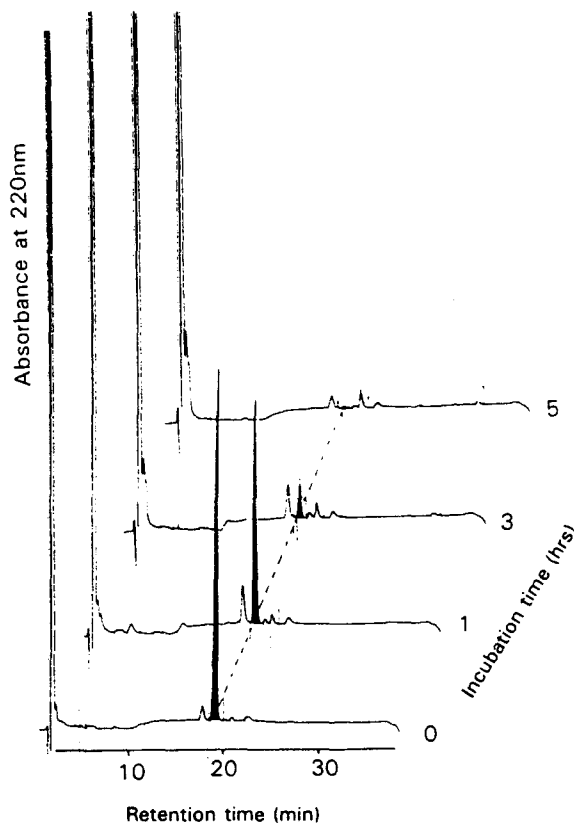


Figure 1. Degradation of the bitter-tasting C-peptide during incubation with *L. acidophilus* 1233 cells in the assay.

Table 1. Debittering activity of several lactic acid bacteria¹

Strain or culture	Debittering activity ($\mu\text{gC-pep} \cdot \text{h}^{-1} \cdot \text{OD}^{-1}$)
<i>L. helveticus</i> T75	0.0
mix-strain culture T57	0.2
<i>L. helveticus</i> T19	1.8
<i>Lactococcus lactis</i> B48	4.6
<i>L. helveticus</i> T96	5.6
mix-strain culture TM5	6.5
<i>L. helveticus</i> B5	6.5
<i>Lactococcus lactis</i> B65	7.3
<i>L. helveticus</i> T172	18.5
<i>L. helveticus</i> T100	19.0
<i>L. acidophilus</i> T74	20.0
<i>L. helveticus</i> T18	34.0
<i>L. acidophilus</i> I233	38.0

¹ Debittering activity is expressed as the decrease of C-peptide per hour.

3.4 Cheese trials

In order to test whether results from the bitter assay can be used to predict bitter formation in cheese, cheeses were made with a number of adjunct cultures in conjunction with the mesophilic starter Bos as the acidifying culture. As shown in Table 2, results from the assay can indeed be used to predict the debittering activity of cultures in real cheese.

It is important to note that the effect of culture conditions on the debittering activity of adjunct cultures was not only found in the laboratory assay, but also in the cheese trials. For instance, culture TM5 was found to have an increased debittering activity when grown under pH-controlled conditions. The same culture resulted also in non-bitter cheeses, whereas the reference cheese, prepared with the addition of TM5 grown under acidifying conditions, did result in bitterness (Table 2).

A number of the strains with the highest debittering activity were tested for their ability to debitter cheese made with a starter which is known to develop a strong bitter taste in cheese. As shown in Table 3, selected strains with high debittering activity were indeed able to significantly reduce bitterness in such cheeses. These results show the usefulness of the laboratory assay in the selection of debittering strains and in growth conditions of adjunct cultures by which bitterness in cheese can be controlled.

It is noteworthy, that the debittering activity of the assay should not be used as an absolute and only characteristic for the characterization of strains with respect to their debittering capacity, since it cannot be excluded that peptides other than the C-peptide contribute to the bitterness of cheeses (2). Moreover, compounds other than peptides might contribute to or enhance the bitterness (14). This might also explain the strain-dependency which was observed for some of the strains in the cheese trials (data not shown). In such cases, bitter-tasting peptides other than the C-peptide might have been released in the cheese or degradation products of the C-peptide might still be large enough to result in a bitter taste.

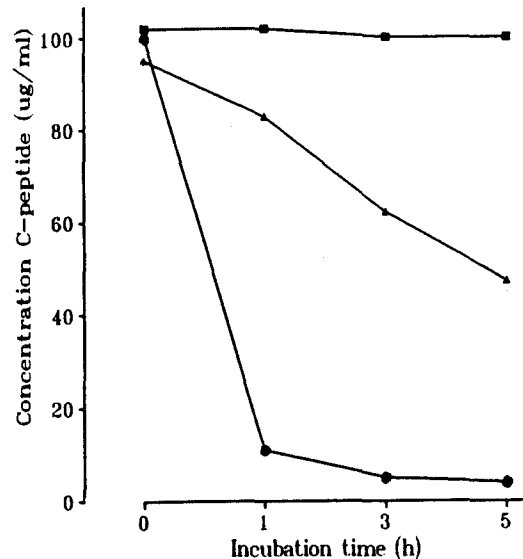


Figure 2. Influence of growth conditions on debittering activity of culture T57.
 ■ acidifying; ▲ pH-controlled; ● acidifying/lysed

Table 2. Debittering activity of thermophilic adjunct cultures in the assay and in cheese after three months of ripening.

Starter culture	Adjunct culture	Growth conditions ¹	Debittering activity ²	Bitter score in cheese ³
Bos	T19	acidifying	1.8	1.7
Bos	T172	acidifying	18.5	< 0.1
Bos	I233	acidifying	38.0	< 0.1
Bos	TM5	acidifying	6.5	1.9
Bos	TM5	pH-controlled	10.1	0.1

¹ Cultures were grown for 40 h under acidifying or pH-controlled conditions at pH 5.7.

² Debittering activity of adjunct cultures determined in the bitter assay (see Table 1).

³ Bitter score on a scale of 0 (absent) to 4 (very strong).

3.5 Effect of growth conditions on production of volatile flavour components

Volatile flavour components in cheese were determined in order to establish whether growth conditions of the adjunct culture might affect the formation of other flavour components as it affects the debittering activity. For this purpose cheeses made with culture TM5, grown under different growth conditions, were examined, since there was a clear difference with respect to bitterness in these cheeses (Table 2). However, when the same cheeses were analyzed for volatile flavour components, no significant difference could be detected (Fig. 3). Organoleptic evaluation of the cheeses corroborated the latter results, since, apart from the bitter score, the cheeses were judged similarly. Therefore, for this culture, the effect of growth conditions apparently does not strongly affect the formation of volatile flavour components.

In conclusion, the bitter assay allows a fast screening for and predicts of the debittering activity of lactic acid bacteria. The mechanism which accounts for the differences found among strains might be a result of differences in (sensitivity) to lysis under cheese conditions. Bitterness in cheese can be controlled by adaptation of the growth condition and/or by the use of highly debittering strains as adjunct culture.

Table 3. Influence of *L. acidophilus* I233 on the development of bitterness in cheese after 3 months of ripening

Starter culture	Adjunct culture	Bitter score in cheese ¹
Bos/T72	none	2.7
Bos/T72	I233	0.2
13M/C17	none	1.5
13M/C17	I233	0.1

¹ Bitter score on a scale of 0 (absent) to 4 (very strong).

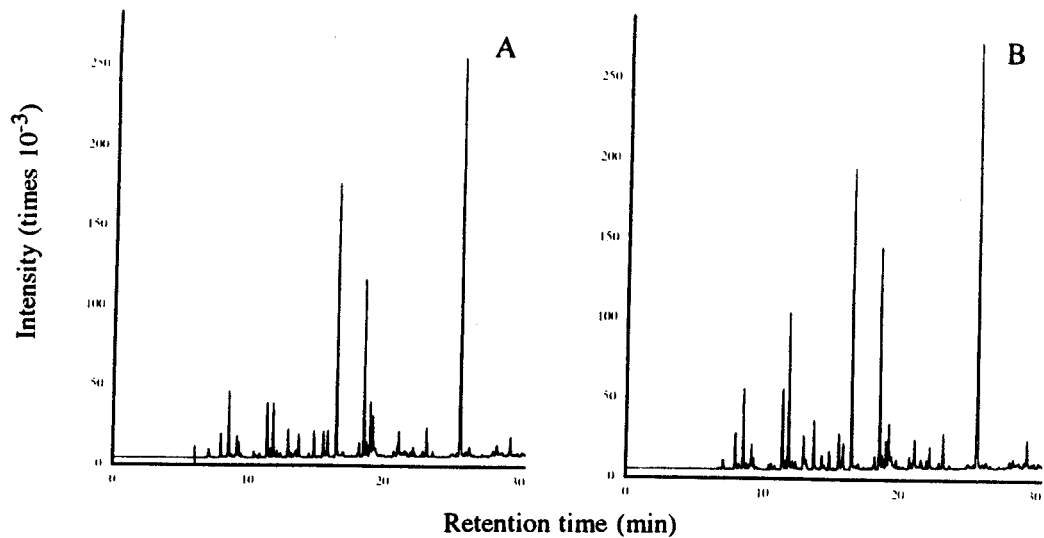


Fig. 3. GC-Aromagrams of 3-month old cheeses prepared with culture TM5 grown under acidifying (A) and pH-controlled (B) conditions.

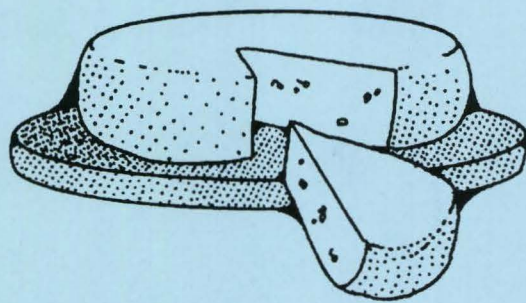
ACKNOWLEDGEMENTS

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**CULTURE METABOLISM
&
CHEESE FLAVOR**



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INTRODUCTION

Lower fat cheese requires specific organisms which lack the ability to produce off-flavors and contain the ability to produce more desirable flavors, however full fat cheese production tends to ignore these starter characteristics since some defects are masked by fat cheese. Unfortunately, flavor defects are intensified in lower fat cheese and hence selection of starter cultures is very important to produce a quality product. Our lack of understanding regarding the influence of starter cultures in flavor development is highlighted by the short list of important characteristics - which is dominated by fast acid production, bacteriophage resistance, and lack of off-flavor production - for full fat cheese production. While low fat cheese production also requires bacteriophage resistance, a different set of selection criteria and modification to cheese making procedures are required to produce an acceptable lower fat Cheddar cheese.

Studies of cheese flavor and texture development reveal these properties are largely the result of microbial activity in aging curd (Reiter and Sharpe, 1971). Schormüller (1968) found purely chemical reactions to be limited in cheese due to the conditions and considers the enzymes from bacteria to cause the majority of changes associated with cheese ripening. Biochemical reactions in cheese ripening which lead to cheese flavor and texture development primarily include glycolysis, proteolysis, and lipolysis. Undefined secondary reactions, such as protein dephosphorylation and amino acid (AA) metabolism, are important as well (Fox et al., 1993; Reiter and Sharpe, 1971; Schormüller, 1968). Analysis of microbial enzymes in cheese maturation indicate microbial hydrolysis of milk proteins strongly influence cheese flavor development in the non-volatile, water-soluble fraction containing compounds with molecular weights below 1000 (Adda et al., 1982; Ardo et al., 1989; Aston and Creamer, 1986; El Soda and Pandian, 1991; Fox et al., 1993; Law, 1987; McGarry et al., 1994; Olson, 1990; Seitz, 1990). As a result, considerable interest has focused on the enzymology and molecular biology of lactococcal proteinases and the impact these enzymes have on desirable flavors and bitterness (Kok, 1990; Olsen, 1990; Thomas and Mills, 1981). Additionally, free AA's are thought to be precursors for background cheese flavor compounds, but the mechanisms and exact compounds are largely undefined (Adda et al., 1982; Law, 1987 & 1992; McGugan, 1975; Olson, 1990). The literature is full of lists of compounds isolated from cheese, however few have been traced to their origin via a mechanistic route which has led to Fox et al. (1993) to point out the disappointing progress in this area since the 1950's.

Secondary proteolysis reactions, such as AA degradation, play a major role in cheese flavor theories, but the mechanisms by which AA's are transformed to cheese flavor are left undefined. Cheese flavor development theories are dominated by the Single Component hypothesis and the Balanced Component theory (Fox et al., 1993). The single component theory accounts for various cheese flavors by production of a single "cheddar-type" flavor compound, while the Balanced Component theory claims many compounds are involved in flavor and it is the balance or ratio between the components which produces proper cheese flavor. A single "cheddar-type" flavor compound has not been isolated while multitudes of compounds have been found that when mixed give a cheddary-type aroma and flavor - hence the Balanced Component theory is widely accepted. The role of starters in these theories are basically the same - they produce acid, die during aging, and lyse to release their intracellular enzymes into the curd which in turn increase proteolysis via peptidase activity. On first inspection these series of events could be correct since cell numbers appear to decrease in aging curd. However, on further inspection it is

possible to formulate an alternative explanation – other than starter death, lysis, and peptidase activity – which is documented in microbes living in harsh environments. The conditions in cheese curd are very harsh for bacteria considering a pH of 5.2, 5% salt in the moisture, a temperature of 4 to 10°C, and <0.01% sugar is present initially by two days after manufacture. Additional support for this shift away from the death and lysis model is that many of the enzymes which play a role in flavor development are not active in cheese curd, peptidase activities don't increase in the curd during aging, and metabolic shifts occur in cultures when grown in cheese slurries which change the AA pool during aging.

Most investigators agree peptidases are important to cheese flavor development, but little is known about the relative influence of individual peptidases (Fox et al., 1993), and even less attention has been given to the secondary AA catabolic reactions which warrant further study before genetic manipulations can be used to improve specific flavor producing pathways (Steele and Ünlü, 1992). While free AA's have been linked to cheese flavor intensity and levels change during aging, over expression of the predominant aminopeptidase (*pepN*) in lactococci do not improve cheese flavor, but high *pepN* activity in cheese curd will debitter cheese (Christensen, 1995; McGarry et al, 1994), suggesting additional mechanisms beyond aminopeptidase activity, free AA's, and peptides are responsible for cheddar-type flavor development.

MORE THAN PROTEOLYSIS?

Important metabolic shifts which impact cheese flavor is evident by the lack of predictive laboratory criteria used in strain selection and large reliance on empirical information from cheese manufacture to confidently select cheese making strains. Industrial observations indicate the best aged Cheddar cheese, lower fat and full fat, is most often made with *Lactococcus lactis* ssp. *cremoris* which is less salt tolerant and unable to degrade Arg to ammonia in the laboratory (Sandine, 1985). These two traits are thought to cause this subspecies to die and lysis rapidly during aging releasing the intracellular contents, specifically the meriade of intracellular peptidases, into the curd leading to increases in total proteolysis and more intense Cheddar flavor (Fox et al., 1993). Release of intracellular enzymes into the cheese matrix to increase cheese flavor has been the dogma for the past half century and has lead to the assumption that cells die and lyse during aging; however, apparent cell death does not necessarily parallel lysis and release of intracellular contents in lactococci (Krishna and Dutta, 1976). In a variety of other areas of microbiology cells have been observed to give the appearance of death by traditional growth parameters, and yet continue to be viable. This leads to the observation that the biochemistry of the medium continues to change even though the cultures is "dead" by traditional measures. This observation in other areas of microbiology parallels ripening cheese (Weimer, unpublished data).

Alternative to observed decreases in lactococci, lactobacilli grow during cheese aging at the expense of peptides and AA's (Peterson and Marshall, 1990). Lactobacilli are typically fastidious in their AA nutritional requirements which force them to produce many proteolytic enzymes allowing hydrolysis and uptake of peptides and AA's in cheese, which is evident in the relatively higher protease activity of lactobacilli as compared to lactococci (Oberget et al., 1991). These characteristics in combination with increased acid tolerance, allow lactobacilli to commonly constitute the non-starter lactic acid bacteria

(NSLAB) in cheese during ripening often growing to 10^8 cfu/g by 6 mon. Even though NSLAB grow to high numbers during ripening their role in cheese flavor development is controversial (Peterson and Marshall, 1990), but some regard these organisms as a major player in cheese flavor (Fox et al., 1993). Oberg et al. (1991) demonstrated lactobacilli vary in their ability to metabolize AA's during growth, however most work has focused on the primary proteolytic capabilities. Recently, *Brevibacteria linens* and *Lactobacillus helveticus*, selected for flavor characteristics, were added to 60% reduced fat Cheddar cheese where both cultures significantly improved flavor acceptance by trained and consumer sensory evaluation (Weimer et al., 1996).

Metabolic characterization of lactobacilli to account for their growth has been attempted to a limited degree. Thomas (1979) and Thomas et al. (1979) observed homofermentative lactococci and lactobacilli to become heterofermentative during carbohydrate limiting growth conditions, indicating conditions in cheese cause metabolic changes in lactic acid bacteria (LAB) that are not normally observed in laboratory conditions. Lactobacilli also produce formate (known to occur in cheese) from citrate in the absence of fermentable carbohydrate (Fryer, 1970). Amino acid analysis revealed all AA's decreased except Ala during sugar starvation and citrate utilization. These data suggest LAB undergo fundamental metabolic shifts during carbohydrate starvation to produce compounds which impact cheese flavor from either AA's or organic acids (lactate or citrate) degradation.

Once peptides or AA's are dephosphorylated and transported into the cell, the general AA catabolic reactions are divided into decarboxylation, transamination, oxidative deamination, and degradation (Hemme, 1982). These reactions produce amines, ammonia, aldehydes, alcohols, organic acids, α -keto acids, phenols, pyrazines, methanethiol, and indole (Hemme, 1982; McGugan, 1975; Schormüller, 1968; Urbach, 1996). Specific compounds from each of these classes are implicated in cheese flavor and thought to be derived from AA's, but exact flavor compounds generated from specific AA catabolism is lean at best (Table 1). Important products or intermediates of AA degradation in cheese are α -keto butyrate, α -keto glutarate, *n*-hydroxyphenyl pyruvate, or pyruvate which ultimately lead to organic acids, other AA's, or other compounds (Ono, 1992; Schormüller, 1968). However, experiments conducted in slurries with added co-factors for AA decomposition increase desirable flavor components with a concomitant decrease in specific AA's (Schormüller, 1968). For example, Ser, Thr, and Arg all decrease with aging to α -amino butyric acid, α -Ala for Ser and Thr. Arg catabolites include citrulline, ornithine, and putrescine. If pyridoxal phosphate is added their degradation increases. This short list of compounds nowhere near matches the known compounds (>150) found in cheese that impacts flavor. But, general classes of compounds include peptides, acids, carbonyls, aldehydes, ketones, mercaptans, amines, and ammonia. These data suggest that secondary AA catabolism may have a significant impact on cheese flavor development and further work is needed to define how to control these reactions for a consistent quality finished product.

METABOLISM & STRESS

Secondary reactions, which use AA's as substrates, involve biochemical pathways allowing the starter culture to contribute to cheese flavor in unique ways that are not evident when they are grown in ideal laboratory conditions. In some cases, such as Arg, the pathway is well known and has been studied in lactic acid bacteria. Other secondary AA catabolic pathways which lead to desirable flavor compounds are uncharacterized in lactic acid bacteria. In addition, aging cheese curd is a very harsh environment for microbes which changes bacterial metabolism (Thomas, 1979; Thomas et al., 1979). A factor known to control flavor changes is pH, with AA catabolism being controlled extensively by this parameter. As such, AA concentration has been used to follow or predict cheese flavor with limited success. A complicating factor in this approach is AA's are interconverted to other AA's with common intermediates being pyruvate and α -keto acids (Schormüller, 1968). One mechanism to achieve this interconversion is transamination with α -keto butyrate or α -keto glutarate and various other intermediates to form new AA's. Schormüller (1968) found Asp, Val, Met, γ -amino butyrate, Phe, Leu, and Ile to be reaminated during cheese ripening via transamination reactions causing a flavor change. This leaves metabolic products from AA catabolism largely undefined by comparison to casein degradation - yet these biochemical reactions lead to flavor compounds significant in cheese flavor.

Past AA catabolism studies are limited and have focused on Arg and Met transformation (Fox et al., 1993; Lindsey and Rippe, 1986). Met degradation has been a focus area of relating AA catabolism to cheese flavor and is considered a desirable microbial end product associated with cheddary-type sulfur notes from the production of methanethiol which increases during aging of good quality Cheddar cheese (Fox et al., 1993; McGugan, 1975). A key link to Met degradation is the ubiquitous, essential co-factor pyridoxal phosphate, which can be generated by de novo synthesis in some organisms and is used extensively in many AA degradation reactions (Bergel et al., 1962; Dempsey, 1987; Weimer et al., 1996; Schormüller, 1968; Zhao et al., 1995). It is involved in degradation of Ser, Thr, Met; improves flavor in slurry systems; and participates in decarboxylation and transamination reactions (Schormüller, 1968). In addition to pyridoxal phosphate, addition of other common co-factors, such as glutathione, cobalt, manganese, and riboflavin, also improves cheese flavor (McGugan, 1975).

Lipolytic activity and interconversion of AA's with fatty acids (FA) is also an important feature of starter cultures. However, this area of cheese flavor is extensively understudied. Important reactions include primary lipolysis which releases free FA's and secondary degradation by microbes, especially yeast and mold. This link to cheese flavor has been exploited with the addition of lipolytic enzymes in enzyme modified cheese preparations and accelerated ripening strategies. The draw back in adding enzymes directly is the lack of control. This uncontrolled hydrolysis usually leads to over ripening and off flavors. Therefore, for hard cheeses this parameter maybe of use in a culture selection scheme. But due to lack of defined study the exact selection criteria remain undefined.

Table 1. Flavor-related products from specific AA catabolism in lactic acid bacteria.

Amino Acid	Flavor-related Product(s)	Reference
Arg	Orn, NH ₄ , putrascine	Poolman, 1993
Met	CH ₃ SH, <i>n</i> -butyrate	Law and Sharpe, 1978; Schormüller, 1968
Glu	4-aminobutyrate	Weiller and Radler, 1976
Orn	putrascine	Cunin et al, 1986, Adda, 1982
Cys	H ₂ S	Fox et al., 1993
Phe	phenylacetaldehyde, phenethanol	Fox et al., 1993
Tyr	<i>p</i> -cresol, phenol, <i>p</i> -hydroxybenzoate	Fox et al., 1993; Schormüller, 1968
Trp	indole	Lindsey, 1994
Leu	3-methyl butanal, valerate	Fox et al., 1993; Schormüller, 1968
Ile	2-methyl butanal, valerate	Fox et al., 1993
Val	2-methyl pentanal, α -keto isocaproate	Fox et al., 1993; Schormüller, 1968
Ala	acetaldehyde, CO ₂ , diacetyl, propionate	Harper and Wang, 1980; Schormüller, 1968
Lys	cadaverine	Schormüller, 1968
Ser	α -amino butyrate, propionate	Schormüller, 1968
Thr	α -alanine, <i>n</i> -butyrate	Schormüller, 1968
Asp	malic acid, oxaloacetate, propionate	Schormüller, 1968
Glu	<i>n</i> -butyrate	Schormüller, 1968

Studies relating AA catabolism, beyond Arg and Met, to specific desirable flavor compounds are limited. Investigations of unclean flavors from aromatic AA's of these pathways are slightly more manageable than good flavors because the offending compounds have been identified, isolated, are fewer in number than beneficial compounds, and can easily be quantitated. *p*-Cresol, phenol, indole, skatole, and phenethanol have all been linked to aromatic AA catabolism, but the exact mechanism of production is controversial. These data are discussed by Drs. Broadbent and Steele in these proceedings; therefore, they will not be covered here. Other than to say that the enzymic pathways delineated for the production of these compounds in *B. linens*, lactococci, and lactobacilli are not active at pH 5.2 with 5% NaCl added.

In both glycolytic and AA catabolic pathways pyruvate is a key intermediate in many metabolic processes. As such, multitudes of products (organic acids, carbonyls, AA's, and alcohols) are derived from the reaction of pyruvate with other intermediates (Ono et al., 1992). Examples of the connection between AA catabolism important in cheese ripening and the use of pyruvate is limited, however Tyr and Ala have been examined to a limited degree. Tyr catabolism in some Gram-positive bacteria (including arthrobacteria and micrococci) produce pyruvate and succinate via the homoprotocatechuate pathway, which requires NADPH, as well as *p*-cresol which is linked to off-flavors. Ala catabolism in slurries found the catabolic enzymes are present in starter cultures to produce pyruvate and many other compounds

(α -keto acids, aldehydes, dicarbonyl, and carbon dioxide) (Harper and Wang, 1980). They also concluded metabolic pathways shifted to energy yielding mechanisms during ripening, with α -keto glutarate accumulating in later stages of ripening. These data suggest either transamination reactions slowed, or the substrates involved in these reactions were not present, or AA catabolic intermediates were shifted to energy production. Additionally, these data offer a third piece of evidence that a fundamental cellular switching event occurs in starter cultures during ripening linking flavor development to AA catabolism in live bacteria.

SO MUCH FOOD AND NOTHING TO EAT

Lack of sugar as an energy source during growth leads to starvation conditions, forcing the cell to shift its metabolism to other available nutrients such as AA's (Morita, 1993). Lactococci are prompted by carbohydrate starvation to shift their metabolism from glycolysis to alternate energy sources, namely AA's, allowing them to be metabolically active (Kunji et al., 1993; Thomas and Batt, 1968). Oliver (1993) found *Vibrio* is viable, but non-culturable (VBNC) in non-ideal environments, meaning cells are able to metabolize, but are non-isolatable on rich, non-selective media at optimum growth conditions leading to the observation that the cells die on isolation with plate count agar, but metabolic changes occur in the test tube containing a "dead" culture. These observations are analogous to those for starter cultures during cheese aging and those observed by Krishna and Dutta (1976) for lactococci and lactobacilli in starvation conditions (Weimer, unpublished data). The induced VBNC state, due to sugar starvation and harsh environments, has been observed for a variety of organisms including species from *Vibrio*, *Shigella*, *Pseudomonas*, *Legionella*, *Klebsiella*, *Enterococcus*, *Escherichia*, *Campylobacter*, *Aeromonas*, *Brevibacterium*, *Arthrobacter*, *Micrococcus*, and others (Boyaval et al., 1985; Oliver, 1993). During the VBNC state cells continue to import AA's, metabolize, and yet become non-isolatable in agar media. *Shigella dysenteriae* becomes VBNC and continues to transport and incorporate Met into cellular proteins (Rahman et al., 1994), suggesting AA transport may occur in cheese as well. In addition to sugar starvation, a cellular metabolic shift is documented in response to harsh environments such as temperature shifts away from the optimum, increased salt concentration, and aeration. All these conditions exist during cheese manufacture and promote a starvation response, the VBNC state, in bacteria during cheese ripening (Thomas and Batt, 1968).

The starvation response has been studied to a limited amount in LAB with Thomas and Batt (1968) first reporting on survival of lactococci during carbohydrate starvation and noted that Mg^{2+} , AA's (particularly Arg) change in the growth medium. Lower incubation temperatures protected and prolonged survival in starvation conditions. Thomas (1979) also found homofermentative strains of LAB become heterofermentative after carbohydrate exhaustion in the medium, indicating a fundamental metabolic shift occurs, as seen by others. Kunji et al. (1993, and Chou and Weimer (1996) found *L. lactis* ssp. *lactis* ML3 metabolically active for long periods of carbohydrate starvation - confirming the initial work of Thomas and Batt (1968). Additionally, their studies found cells resuscitate with the addition of small amounts carbohydrate (galactose) or Arg during starvation. Further, they characterized protein expression and found some cellular proteins are degraded while expression of at least 15 new proteins are induced

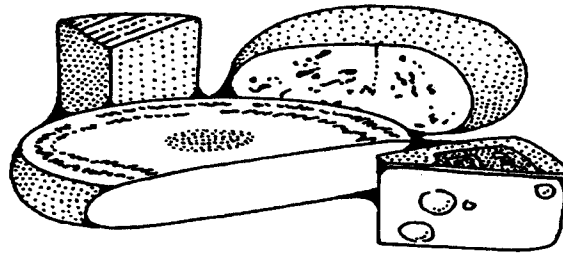
during starvation, suggesting a fundamental metabolic shift occurred to give the cells new capabilities not seen during growth in optimum laboratory conditions. Carbohydrate starvation induces *L. lactis* ssp. *lactis* IL1403 to produce new proteins responsible for cross protection against heat, ethanol, acid, osmotic, and oxidative stress which is a common feature of stress responses. Chou and Weimer (1996) also found that the coordinated expression of the Arginine deiminase pathway has a significant impact on survival. Micrococci, common isolates from aged cheese, also enter a VBNC state and remain metabolically active for 3 to 6 mon of incubation in carbohydrate starvation conditions (Votyakova, 1994). Survival in the VBNC state is associated with the loss the ability to form colonies on solid agar, ability to grow in broth, and reduction in cell size (Votyakova, 1994). All these indicators are seen in lactococci and micrococci during carbohydrate starvation. If we assume starter cultures become VBNC in the cheese matrix three questions arise:

- 1) by what mechanism is the cell sensing starvation?,
- 2) what new proteins and substrates are being induced to remain metabolically active (*i.e.* generate energy in the form of ATP and recycle reducing equivalents)?, and
- 3) what role does this play in cheese flavor?

Once the cell "knows" to begin using other nutrient sources Arg becomes immediately interesting for cheese research because it is used as an energy source in sugar depleted laboratory media, is used as a significant distinguishing characteristic between *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*, yields ATP, recycles NADPH, disappears during cheese ripening, produces compounds associated with desirable cheese flavor, and is induced in *L. lactis* ssp. *cremoris* during starvation (Poolman, 1993; Schormüller, 1968; Thompson et al, 1986; Chou and Weimer, 1996). Other common stress responses cause the cell to accumulate compounds from the environment that protects the cell from further damage. Compounds that are commonly accumulated include Gly, Pro, carnitine, and betaine to combat the effects of osmotic damage (Verheul et al, 1995; Kets et al., 1995). *Lactobacillus plantarum* exhibits this protective mechanism which allowed the cells to grow in 1 M NaCl (Kets et al., 1995). Oxidative stress also causes media components to accumulate need compounds, such as glutathione. Fernandez and Steele (1993) and Wiederholt and Steele (1994) found glutathione to be accumulated by *L. lactis* ssp. *cremoris* while *L. lactis* ssp. *lactis* lack this ability. Accumulation followed the growth curve and acid production, suggesting lactococci accumulate glutathione to combat oxidative stress as they grow. They hypothesized this ability plays a role in the difference in *L. lactis* ssp. *cremoris* and *L. lactis* ssp. *lactis* to produce acceptable aged cheese, which is reasonable since glutathione plays a beneficial role in sulfur compound generation in cheese making and *L. lactis* ssp. *cremoris* is known to produce better flavored cheese than *L. lactis* ssp. *lactis* (Schormüller, 1968; Samples, 1985; Singh and Kristoffersen, 1970).

CONCLUSIONS

Current dogma states starter population die and lyse, but the level of remaining "healthy" cells cannot account for the extensive biochemical changes observed in good quality cheese (Fox et al., 1993; Law and Sharpe, 1978). However many pieces of evidence, when taken together, argue cells don't die and lyse, but rather remain metabolically active to influence cheese flavor. This may explain the observation that most enzymes responsible for enzymic reactions which lead to cheese flavor are not active in the conditions found in cheese curd if added in pure form. If this is the case then many diverse secondary reactions that degrade AA and FA to flavorful compounds can be explained and ultimately controlled. Achievement of this goal will result in products that are consistently more flavorful – for both low and full fat cheese.



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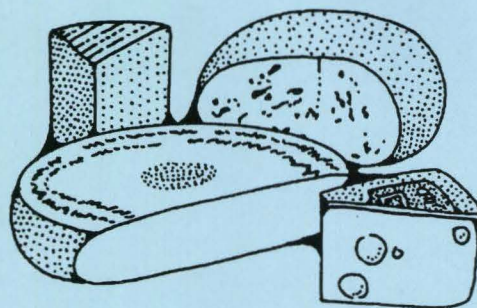
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SWISS CHEESE FLAVOR - FATTY ACIDS AND THE ELECTRONIC NOSE



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Swiss Cheese Flavor - Fatty Acids and the Electronic Nose¹

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This paper will discuss

- an overview of Swiss cheese flavor
- lower molecular weight volatile free fatty acids in low fat and full fat Swiss cheese
- fatty acid flavor thresholds compared to free fatty acids in Swiss cheese
- introduction to the electronic nose
- the application of an electronic nose to determine the significance of free fatty acids to the aroma of Swiss cheese

Flavor of Swiss cheese:

The flavor of cheese is complex, elusive and remains incompletely understood. Over 200 volatile compounds have been identified as potentially contributing to the flavor of cheese. These compounds include fatty acids, alcohols, aldehydes, amines, esters, furanones, ketones, lactones, phenols, pyrazines and sulfur compounds.

The focus of this paper will be on the role of lower molecular weight fatty acids in the flavor of Swiss cheese. Forty years ago Hintz, et.al. (1956) reported that propionic acid and proline were associated with the characteristic flavor of Swiss cheese. Mitchel (1981) confirmed that these compounds simulated a Swiss cheese flavor, but that they did not provide a "full bodied" Swiss flavor.

Much of our knowledge of Swiss cheese flavor results from the work of Hammond and associates, who found acetic, propionic and butyric acids associated with Swiss cheese flavor. Propionic acid was found associated with nutty, sweet and burnt flavor attributes. Later Hammond and Griffith (1989) thought that chemical reaction products between dicarbonyls and amino acids were of special importance to the flavor of Swiss cheese.

Some new methodologies may provide additional knowledge to our understanding of Swiss cheese flavor, especially the aroma portion. Grosch (1994) reported on an aroma extraction dilution analysis, which provides an index of odor activity (ratio of compound concentration to flavor threshold value) and has reported that only a small fraction of the complex mixture of volatiles cause the characteristic aroma of a given food. Preiniger, et.al. used a model system to determine the key compounds that contributed to the flavor of Swiss cheese. The results of sensory panel evaluation indicated that

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combinations in the concentrations found in Swiss cheese of methional, 4-hydroxy, 2,5-dimethyl-3 [2H]-furanone, 2 ethyl, 4-hydroxy-5-methyl 3 [2H] furanone, acetic acid and propionic acid + selected non-volatile compounds provided a product that had similar sensory profiles to Swiss cheese. [The non-volatile components included lactic acid, succinic acid, glutamic acid, ammonia salts and a mixture of cationic and nonionic salts.

Another new methodology of potential value is the Electronic Nose, which will be discussed in detail later in this paper. Let us say at this point that it may provide a means of testing Grosch's hypothesis that only a few compounds really contribute to the aroma portion of cheese flavor.

Free Fatty Acids in Low Fat and Full Fat Swiss Cheese and Relationship to Flavor:

The inferiority of flavor and texture of low fat cheese is well established. Today we will be concerned about the flavor aspects, although we did report at the recent ADSA meetings of approaches to improve the texture of low fat Swiss Cheese

For the purpose of this presentation, low fat cheese shall mean a cheese that has a 50% fat reduction relative to the full fat cheese. Initially we studied 11 pairs of commercial full fat and low fat Swiss cheese made from the same milk and starters on the same day. These cheese were obtained at about 3 months of age. The low fat product had about 10% higher moisture than full fat and a fat replacer was used. The flavor of the low fat products were all poorer than the corresponding full fat control. We sampled a portion from the edge, center and around the eyes. Water extracts were prepared by a modification of Kunchroo and Fox (1984). The lower molecular weight fatty acids (C-2 to C-10) were determined by direct injection (splitless mode) of 0.2 ml of water extract onto a HP GC column (0.32 mm x 25 m). The GC was temperature programmed from 110 to 230°C at 10°C/minute. The internal standard was 4-methyl valeric acid.

The findings were as follows:

- no significant difference was found in the acetic acid levels in full fat and low fat cheeses
- the concentration of propionic acid was about an order of magnitude less in low fat than in full fat cheese
- butyric acid in low fat cheese was about ½ of that in the full fat cheese
- there was no significant difference in the acetic acid concentration in different portions of the sample
- the concentration of propionic and butyric acids was highest around the eyes
- the concentration of the C-6 to C-10 ffa was about 1/10th that of butyric acid

Based on this information, the manufacturer modified the process for making the low fat cheese, including a change in starters. As a result, the differences in propionic acid levels in full fat and low fat cheese was eliminated. Additionally, sensory evaluation indicated a significant improvement in flavor -- indicating that lower molecular weight acids are

significant in Swiss cheese flavor. However, the change in fatty acids did not eliminate the flavor differences between the two types of cheese.

Figures 1, 2, 3, 4, 5, and 6, show the three major ff. in full fat and low fat cheese at 2, 3 and 4.5 months of age for 3 pairs of cheese made by the old and new procedures.

Figure 1: Acetic acid in low fat and full fat Swiss cheese - old method

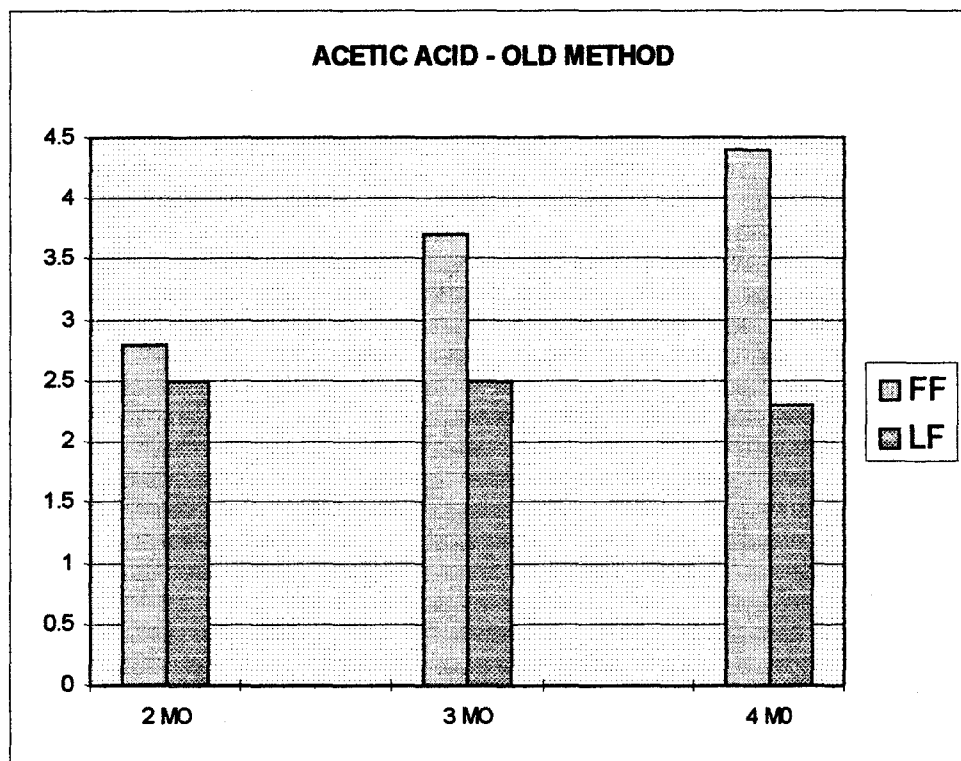


Figure 2: Acetic acid in low fat and full fat Swiss cheese - new

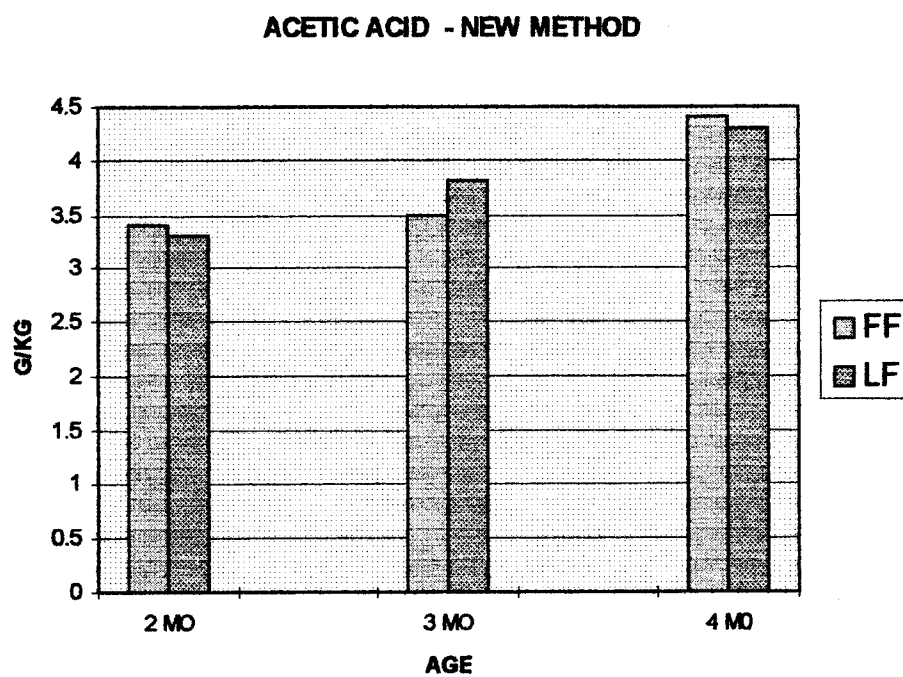


Figure 3: Propionic acid in Swiss Cheese - Old method

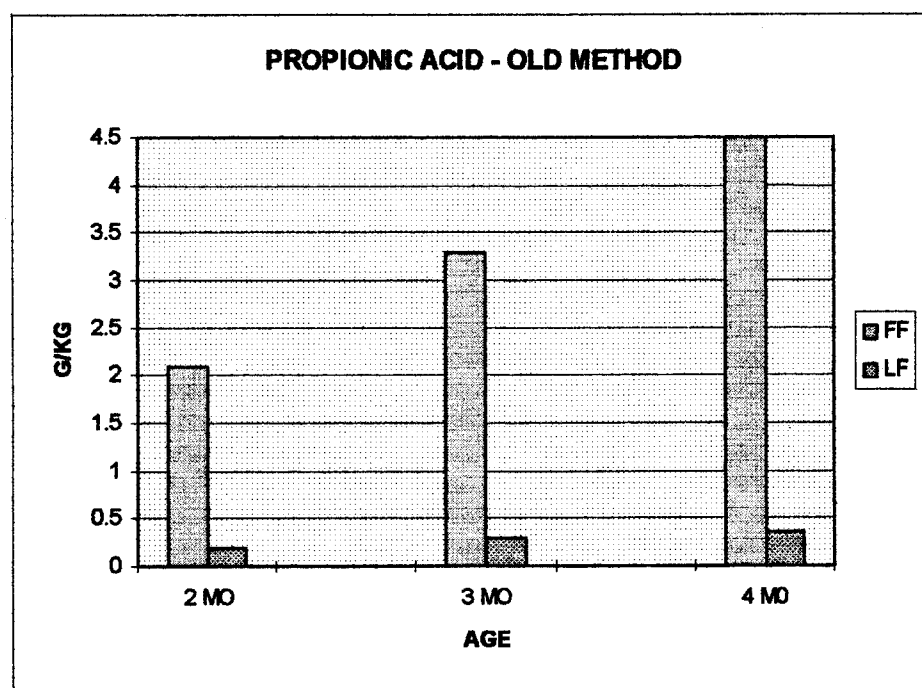


Figure 4: Propionic acid in low fat and full fat Swiss cheese - new method

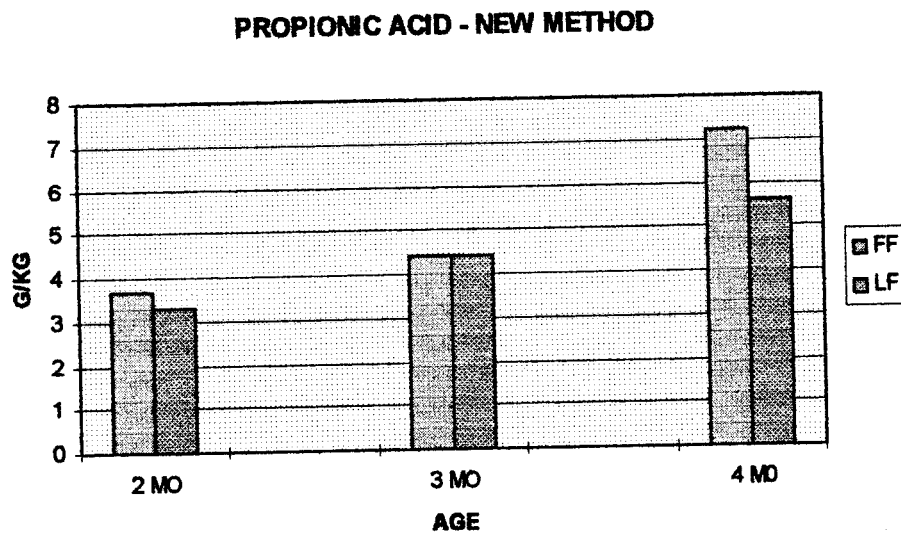


Figure 5: Butyric acid in low fat and full fat Swiss cheese - old method

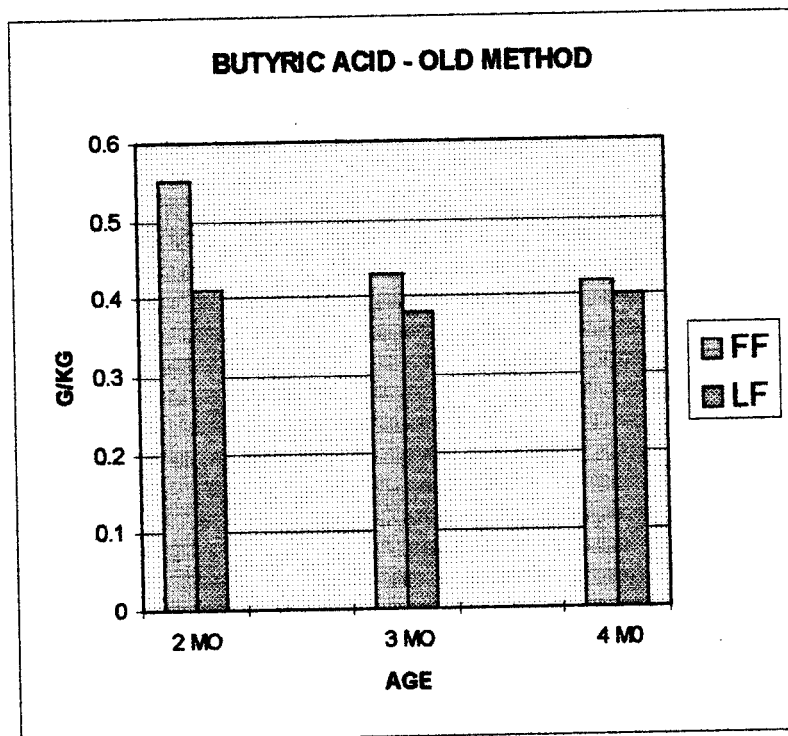
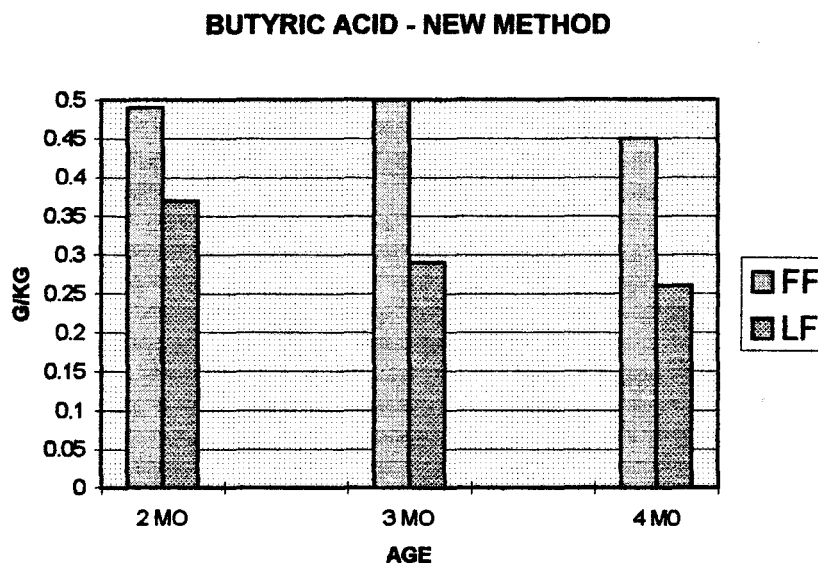


Figure 6: Butyric acid in low fat and full fat Swiss cheese - new method



Acetic acid concentrations in low fat and full fat cheese are about the same at two months of age, for cheese made by both the new and old methods (Figure 1). However, for cheese made by the old procedure, the acetic acid values increased with age for the full fat cheese, but not in the low fat cheese. With the new procedure the acetic acid values were quite similar and increased during ripening to about the same extent (Figure 2)

As shown in Figure 3, the propionic acid levels in the low fat cheese were much lower than those in the full fat by the old procedure. Also the propionic acids concentrations increased more rapidly in the full fat cheese. These marked differences were minimized by the new procedure, although there was significantly less propionic in the low fat than in the full fat cheese at 4.5 months (Figure 4).

Butyric acid did not increase with ripening for either full fat or low fat cheese made by either procedure (Figure 5 and 6). At 2 months there was less butyric acid in the low fat cheese for cheese made by both the old and new procedure.

Fatty Acid Threshold Values Compared to Concentrations in Swiss cheese

Table 1 shows a comparison of human sensory threshold levels for aroma compared to the acid concentrations of the major low molecular weight fatty acids found in low fat and full fat 4 month old cheese.

Table 1: Major lower molecular weight free fatty in 4 mo. Old Swiss cheese compared to published aroma thresholds (ppm)

ACID	AROMA THRESHOLD ₁	FULL FAT	LOW FAT
ACETIC	100	4250	4230
PROPIONIC	40.3	7110	6250
BUTYRIC	0.3	349	250

1. BRENNAND, ET. AL. J. SENSORY SCI. 4:105. 1989

In all cases the concentrations of these acids in this Swiss cheese exceed the reported aroma threshold values by >40 times. [For full fat cheese: acetic acid = 42 X threshold; propionic acid = 175 X threshold; butyric acid = 1160 X threshold]

For the minor lower molecular weight fatty acids listed in Table 2 , only iso-valeric and caproic acids exceeded the published threshold values. [For full fat cheese: iso-valeric = 19 X threshold and caproic = 2 X threshold]. Valeric acid was at about the threshold level, whereas both caprylic and capric acids were present at less that threshold levels.

Table 2: Minor lower molecular weight free fatty acids in 4 mo. old Swiss cheese compared to published aroma thresholds (ppm)

ACID	AROMA THRESHOLD ₁	FULL FAT	LOW FAT
VALERIC	6.5	6	7
ISO-VALERIC	3.2	60	64
CAPROIC	9.2	31	18
CAPRYLIC	19.0	9	5
CAPRIC	2.3	1.1	1.0

1. BRENNAND, ET. AL. J. SENSORY SCI. 4:105. 1989

Introducing the Electronic Nose:

The electronic nose represents a new technology developed to provide an objective method for evaluating the aroma of foods, based on the principles by which the human nose differentiates aroma.

There are a number of electronic noses currently on the market, each of which has an array of multiple semi-conducting sensors -- ranging from 6 to 32. These sensors bind volatile compounds either on the basis of charge, size, hydrophobicity, etc. Upon binding, the resistance of the sensor is changed. The rate of binding and the rate of change in sensor resistance over time is a function of the aroma of the food being evaluated. Each instrument has its own array of sensors, measurement system and data analysis

methodology. Sensors may be metal oxides, organic polymers, acoustic wave or micro-balances and vary in number from 6 to 32.

Data analysis is critical. We use a stepwise approach, which includes determination of sensor validation, significance of fit, untrained and trained differential analysis. If OK, the data can be presented in the form of canonical discriminate analysis.

A detailed description of the various instruments and the factors which influence their efficacy is beyond the scope of today's presentation. What I want to present now is the application of one of the three Electronic Noses in our laboratory to provide a better understanding of the role of fatty acids in the aroma of Swiss Cheese. For this study we have used a n Alpha M.O.S. Fox 2000, supplied with 6 metal oxide sensors. One gram of cheese was used, placed in a sealed container and the aroma assessed at 40°C.

The Electronic Nose has provided us with a basis for:

- differentiating Swiss cheese with difference flavor properties
- assessment of the Electronic Nose threshold for fatty acids in green cheese curd
- the significance of various fatty acids and fatty acid combinations to Swiss cheese aroma

Differentiating Swiss Cheese with Different Flavor Profiles:

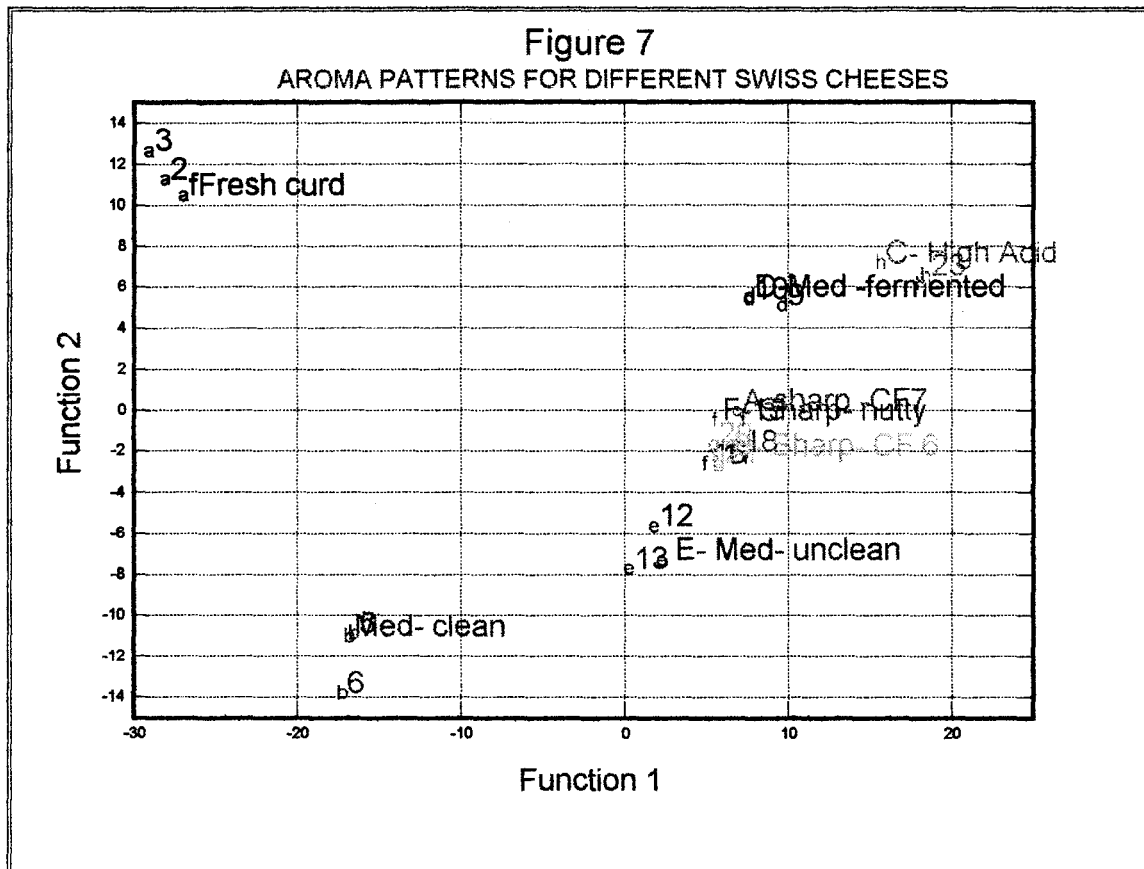
Fresh curd and 7 Swiss cheese samples that had different flavor profiles were evaluated with the Fox 2000 Electronic Nose. The cheeses were different from one another in flavor attributes Table 3.

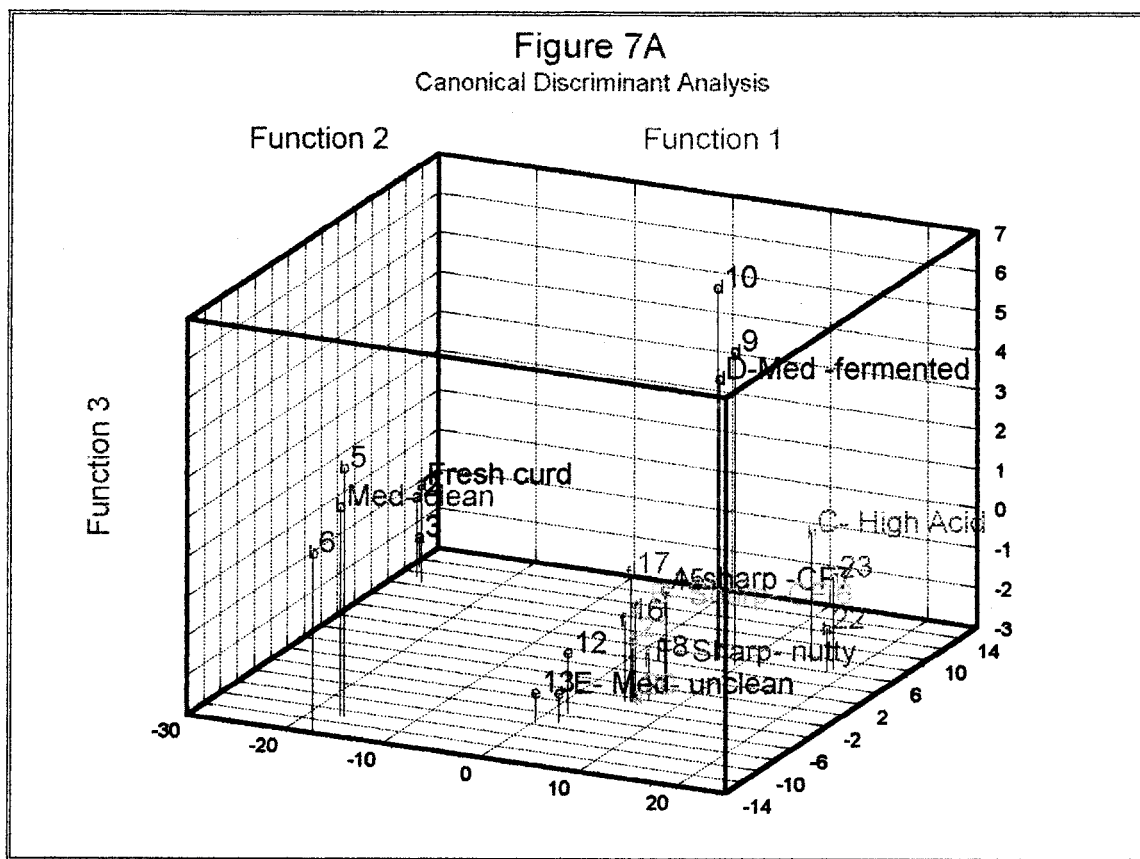
Table 3: Description of Swiss cheese used for aroma analysis.

SAMPLE DESIGNATION	DESCRIPTION	MAJOR FLAVOR CHARACTERISTIC ¹
Curd	Fresh Swiss cheese curd, directly from the vat at dipping	Bland
Med- clean	3 month old Swiss,	No defects, CF=3
A-sharp	>6 month old Swiss	CF=7
B-Nutty	>6 month old Swiss	strongest nutty, CF=5
C-Acid	>6 month old Swiss	Acid - 6, CF=4
D-Med-fermented	>6 month old Swiss	Fermented -6, CF=3
E-Med-unclean	?	Unclean -5, CF=3
F-Sharp	>6 month old Swiss	CF=6

1. CF= characteristic flavor. Scores range from 0-8, with 0 being lowest and 8 being highest.

Canonical Discriminate two dimension and three dimensional plots of the results are presented in Figures 7 and 7A. Major attributes of the different samples are noted in the plots. Each sample is represented by three replicate data points. The distance between the centroids provides a measure of the degree of difference between the samples. The data can not be considered as quantitative. The fresh curd is clearly different than the any of the cheeses. Three sharp cheeses (A, B and F) are not differentiated. Two cheeses with acid and fermented flavors are somewhat close together and separate from the sharp cheese. The three dimensional plot provide some additional information about the differences between the cheese samples.





Fatty acid thresholds in Swiss Cheese Curd:

Various concentration of fatty acids from 1 ppm to the concentrations found in cheese were added to fresh Swiss Cheese curd (pH 6.3) to evaluate the threshold detection levels for the various acids. The acids were added to the cheese curd in a mortar and ground for 30 seconds and then placed in a sealed contained equipped with valves to attach to the Fox 2000 Electronic nose. Every effort was made to obtain uniform mixing. All additions were made in triplicate. The data was analysed by canonical discriminate analysis.

Table 4 shows a comparison of the aroma threshold values reported for these acids compared to those found with the Electronic Nose. For these particular volatile compounds the nose appears to be more sensitive than the human nose. [We know from other work, that this is not true for some other classes of compounds.]

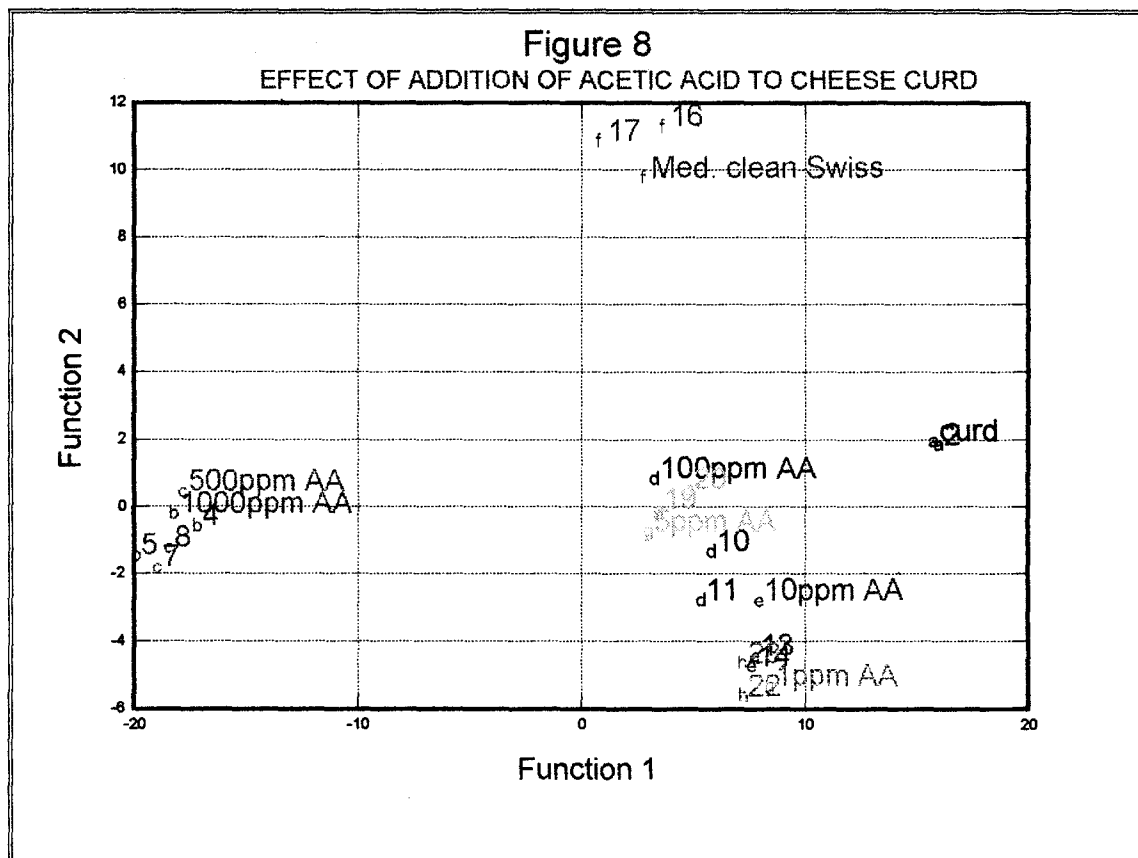
Table 4: Comparison of threshold values in ppm for free fatty acids by a human sensory panel and an electronic nose (fox 2000)

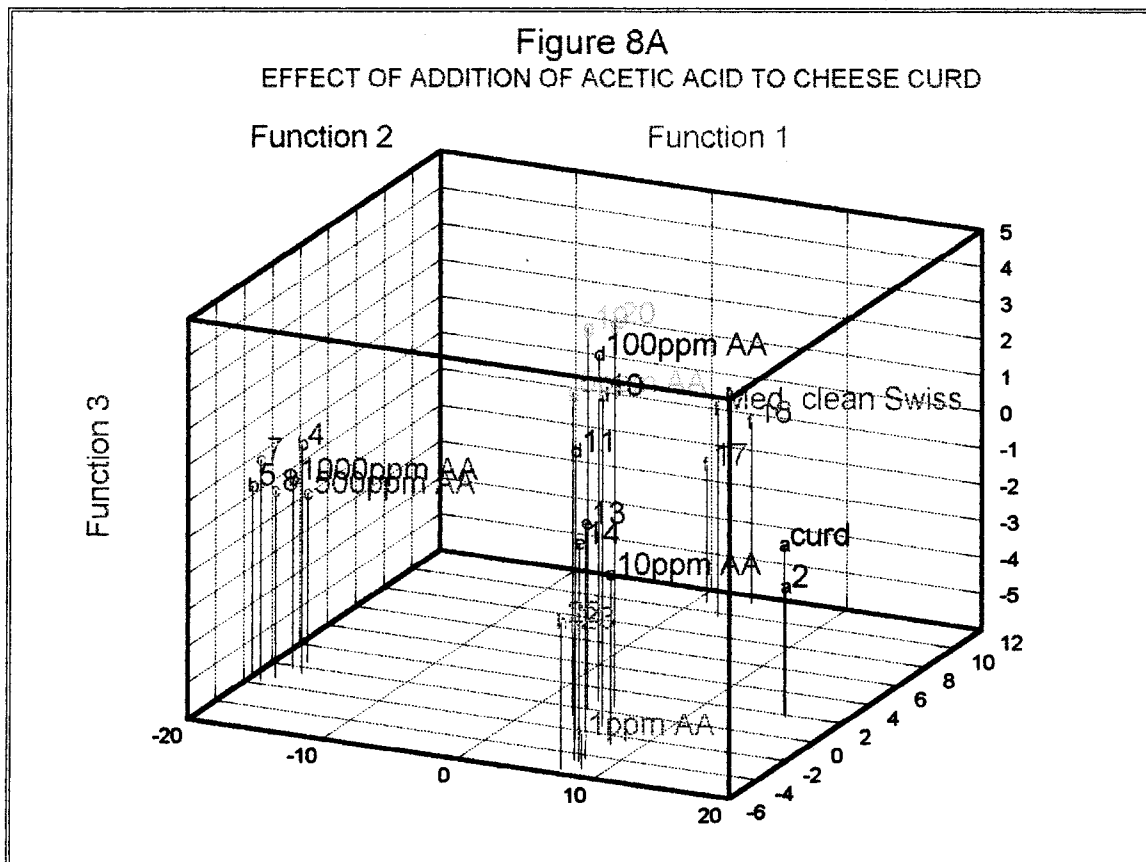
ACID	ELECTRONIC NOSE THRESHOLD	AROMA THRESHOLD ₁
ACETIC	<1	100
PROPIONIC	<1	40.3
BUTYRIC	<1	0.3
VALERIC	<1	6.5
ISO-VALERIC	<1	3.2
CAPROIC	<1	9.2
CAPRYLIC	<1	19.0
CAPRIC	<1	2.3

1. BRENNAND, ET. AL. J. SENSORY SCI. 4:105. 1989

In all cases the threshold values were less than 1 ppm. No attempt was made to find the true threshold values.

Typical data for the addition of various concentrations propionic acid are presented as a two dimensional plot in Figure 8. If the acids were at or below threshold levels, the plots would not be differentiated from the curd.





The Role of Fatty Acids in Swiss Cheese Aroma

Lower molecular weight fatty acids were added individually and in combination to fresh Swiss cheese curd. The closeness of fit provided an indication of the relative significance of the various fatty acids to cheese with different flavor profiles.

Figure 9 presents data on the effect of adding 5000 ppm propionic acid to the fresh cheese curd. The aroma patterns show the relationship of the added propionic acid to the fresh curd and to Swiss cheeses with different flavor profiles. In this case the added propionic acid is close to the medium flavored, clean Swiss – but not to the sharp cheeses. The medium flavored cheese is about 3 months of age. All the other cheeses shown in this plot are >6 months old. This suggests that the major aroma components in the aged cheese are not just propionic acid.

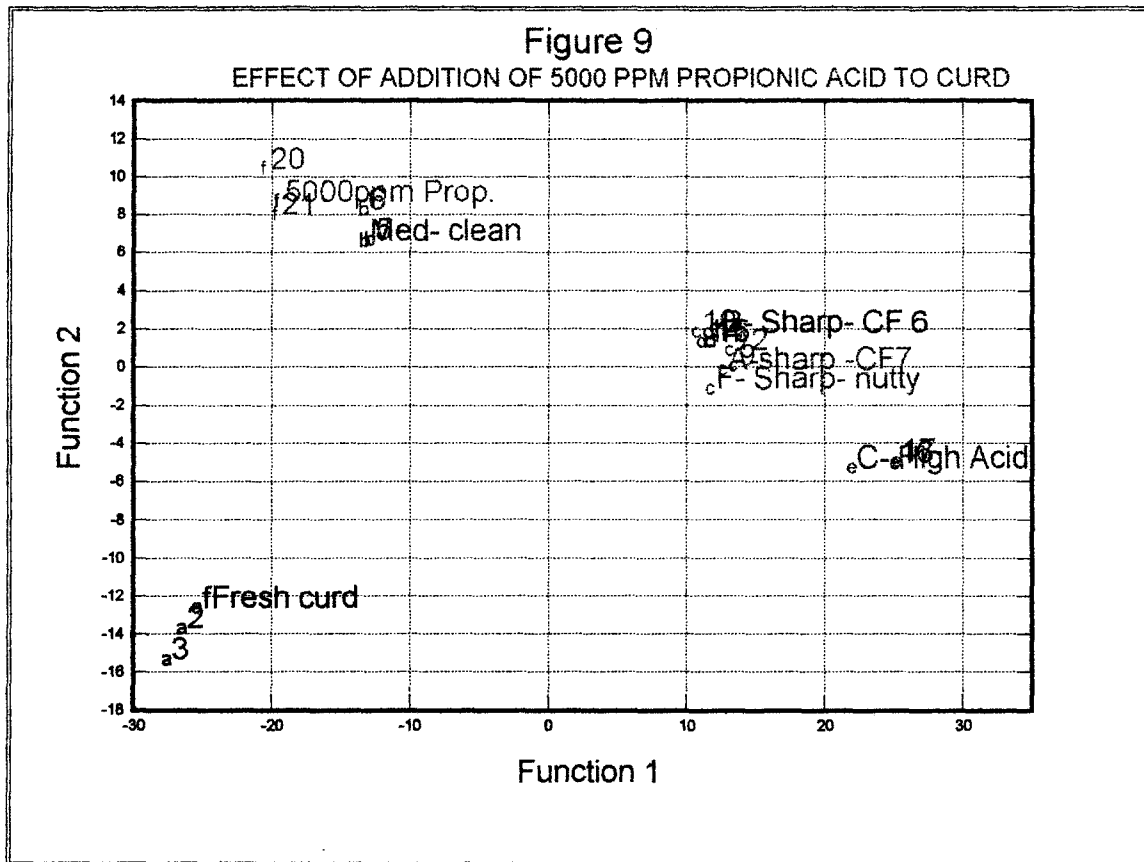


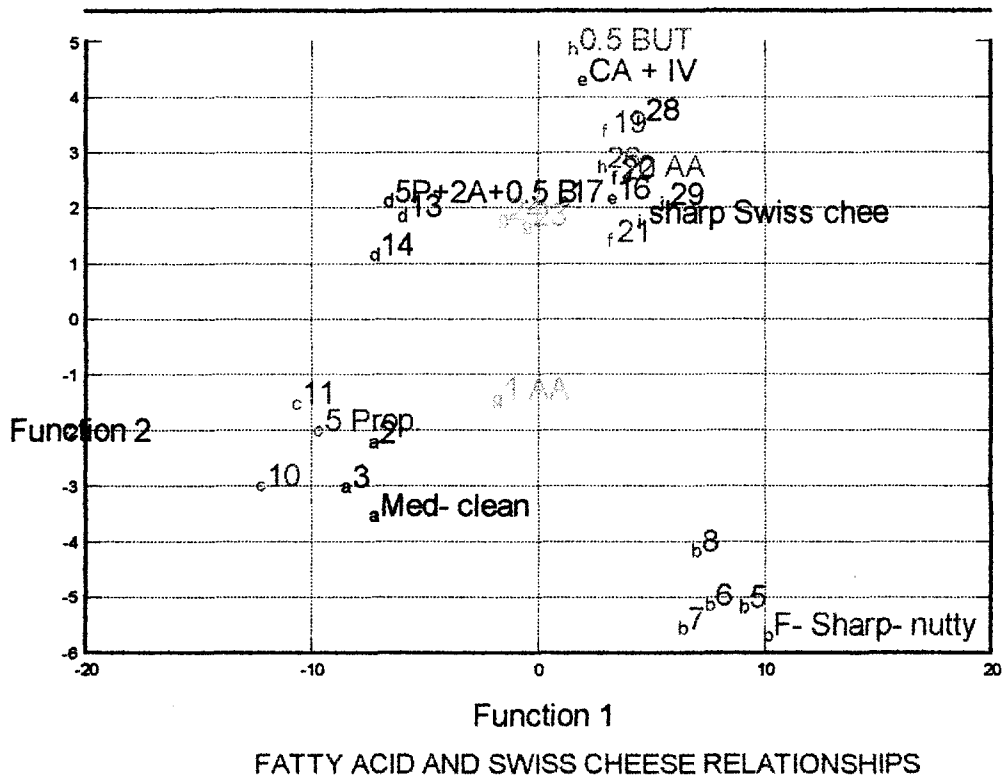
Figure 10 and 10A present the effect of the addition of a variety of acids, singly and in combination to the fresh curd -- in comparison to three Swiss cheeses with different flavors.

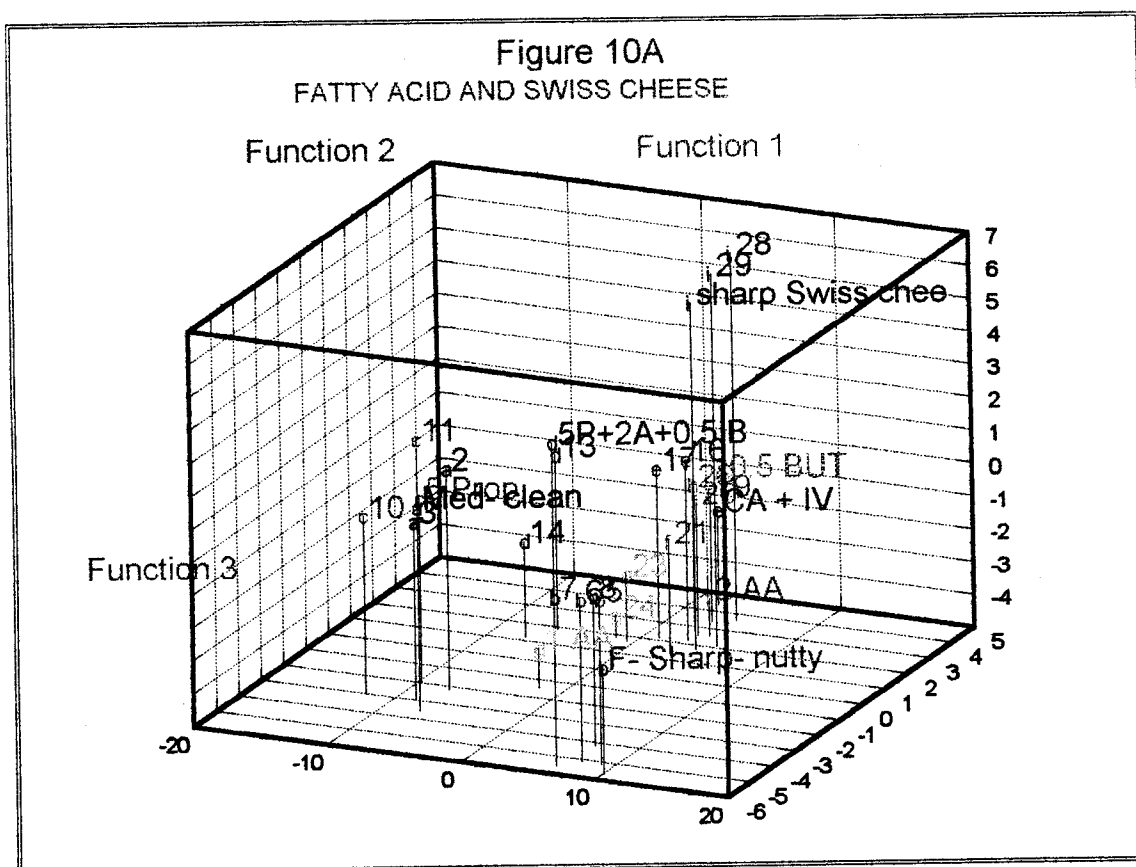
The codes for the acids added are shown in table 5.

Table 5: Description of fatty acids and concentrations added to fresh Swiss cheese curd.

CODE	ACIDS ADDED	AMOUNTS
5 Prop	propionic acid	5000 ppm, 5g/kg
1 AA	acetic acid	1000 ppm, 1 g/kg
2 ASA	acetic acid	2000 ppm, 2 g/l
0.5 BUT	butyric acid	500 ppm, 0.5 g/kg
CA +IV	caproic acid + isovaleric acid	60 ppm caproic + 10 ppm iso-valeric acid
5P+2A+ 0.5B	propionic acid + acetic acid + butyric acid	5000 ppm propionic acid + 2000 ppm acetic acid + 500 ppm butyric acid

Figure 10





Butyric acid, acetic acid and the combination of propionic, acetic and butyric are more closely associated with the sharp cheese than with the medium of sharp nutty acid. However, they do not match – suggesting that other compounds are also involved.

The data does suggest that this approach holds promise for determining the significance of different volatiles to cheese aroma. This will require very careful sensory evaluation and considerable work to move this work forward.

Future work will be directed to determining the role of other compounds in respect to the different flavor attributes of Swiss cheese.

Conclusions:

The following conclusions are proposed.

1. The role of low molecular weight fatty acids to the flavor of Swiss cheese has been reconfirmed.
2. Propionic and butyric acid formation in low fat Swiss cheese is more sensitive to changes in process than for full fat cheese.
3. Butyric acid appears to be formed during warm room fermentation, rather than from

lipolysis.

4. The electronic nose is very sensitive to free fatty acids, with threshold levels of all acid evaluated of <1 ppm.
5. The electronic nose differentiates Swiss cheese with differing flavor profiles.
6. The electronic nose provides an objective method for assessing the relative significance of flavor compounds in cheese flavor
7. Different free fatty acids appear to have different significance in regard to different Swiss cheese flavor characteristics.

Acknowledgments.

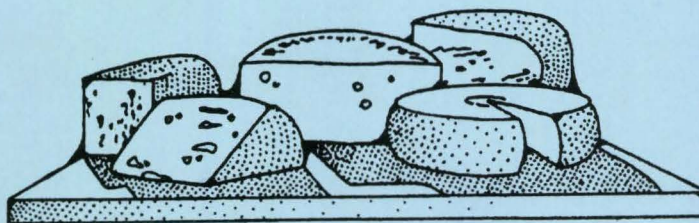
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HOW TO REDUCE BITTERNESS IN LOW-FAT CHEESE



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HOW TO REDUCE BITTERNESS IN LOW-FAT CHEESE

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An area of growing interest in the dairy industry is the production of high quality, low-fat cheeses. Interest in these products is the direct result of market research indicating that many consumers desire to reduce dietary fat intake. However, it has proven difficult to produce high quality low-fat cheeses, especially ripened varieties, to meet this demand. When a significant percentage of fat is removed, rheological properties change and flavor and texture are adversely affected. In general, the lower the fat content, the more difficult it is to produce a cheese similar in quality to full-fat cheese. Common defects include lack of flavor and a texture which is described as curdy and gummy. Low-fat cheeses are also more susceptible to culture-related flavor defects such as bitterness and meaty-broth flavors. The focus of this presentation will be on the compounds responsible for the development of bitterness, the enzymes involved in their formation and hydrolysis, and steps which can be taken to reduce their accumulation in the cheese matrix.

It is well established that bitterness in fermented dairy products is the result of proteolysis of milk proteins leading to the accumulation of hydrophobic peptides in the cheese matrix. The hydrophobic nature of bitter peptides was examined in detail by Ney (7). The primary outcome of these studies was that by simply calculating the average hydrophobicity of a peptide (Q value), the sum of hydrophobicity values of individual amino acids divided by the number of amino acid residues, it should be possible to predict if a peptide will be bitter. The results indicated that peptides with a Q value of +1400 cal/mole or greater were bitter, while peptides with Q values of less than +1300 cal/mole were non-bitter. Peptides with Q values between +1300 and +1400 may be either bitter or non-bitter. Subsequently, Guigoz and Solms (4) examined the relationship between Q value and bitterness with 206 peptides for which information concerning taste was known. Their results indicated that the majority of bitter peptides have Q values greater than +1400 cal/mole; thereby, supporting Ney's hypothesis. Of the bitter peptides that did not fit Ney's hypothesis, many contained glycine, which has no side chain. Guigoz and Solms suggested that glycine should be omitted in the calculation of a peptide's Q value. A more systematic and quantitative approach to peptide bitterness was reported in a series of papers by Belitz and Wieser, which has been summarized in English by Belitz et. al (2). They determined that total hydrophobicity rather than average hydrophobicity more accurately predicts if a peptide will or will not be bitter. Knowledge of the structure of bitter peptides makes it possible to, by using relatively simple calculation, determine whether or not a given peptide is likely to cause a bitter defect in cheese.

Proteolysis in the cheese matrix is a sequential process involving milk-clotting enzymes, milk proteinases (particularly plasmin), the starter culture, secondary microorganisms, and non-starter lactic acid bacteria (3). Chymosin, in addition as serving as the coagulant, is primarily responsible for initiating the degradation of both α_{s1} -casein and β -casein. Hydrolysis of α_{s1} -casein by chymosin takes place primarily at the Phe₂₃-Phe₂₄ or Phe₂₄-Val₂₅ bonds, resulting in a significant softening in the cheese texture. It has been postulated that the resulting α_{s1} -casein (f1-

23/24) peptide is the primary target for the proteolytic enzyme system of lactic acid bacteria and therefore likely plays a central role in cheese flavor development. Hydrolysis of β -casein by chymosin occurs primarily at the Ala₁₈₉-Phe₁₉₀ or Leu₁₉₂-Tyr₁₉₃ bonds. Cleavage at these bonds is particularly significant as the resulting peptides are believed to cause bitterness in cheese. Additional cleavage sites by chymosin on β -casein include Leu₁₃₉-Leu₁₄₀ and Leu₁₆₃-Ser₁₆₄. The peptides generated by chymosin are then further degraded by the proteolytic enzyme systems of starter and non-starter lactic acid bacteria.

The proteolytic enzyme systems of lactic acid bacteria are known to be essential for cheese flavor development and growth of these organisms in milk (3, 8). The lactococcal proteolytic enzyme system is the best characterized and is comprised of proteinases, endopeptidases, and exopeptidases (8). These enzymes function sequentially to hydrolyze casein to amino acids. The lactococcal cell envelope proteinase or CEP has been characterized in great detail. Although lactococcal CEPs exhibit an extremely high degree of amino acid sequence identity, these enzymes can be divided into several classes based on their relative affinity for individual caseins and substrate cleavage sites. Research conducted primarily in the Netherlands has shown that most differences in CEP specificity are due to one or more amino acid substitutions in the enzyme's substrate binding regions. Three distinct endopeptidases, enzymes which cleave peptide bonds within a peptide, have been characterized; while they differ in their peptide bond specificity, all are active only on peptides having between 5 and 30 amino acids. Exopeptidases which have been isolated and characterized include general aminopeptidases, X-prolyl dipeptidyl aminopeptidases (X-PDAP), tripeptidases, and dipeptidases (DP) (14,28,29,31). General aminopeptidases sequentially remove amino acids from the amino terminus of peptides; they have a broad-substrate specificity and are capable of removing a variety of amino acids from the amino terminus of peptides. X-PDAP removes dipeptides from the amino terminus of peptides when proline is in the penultimate position. Tripeptidases cleave tripeptides into dipeptides and amino acids; typically they have a broad substrate specificity. DPs cleave dipeptides into amino acids; both DPs with broad substrate specificity and DPs with narrow substrate specificity have been described. Prolidase is an example of a DP with a narrow substrate specificity, only dipeptides which have proline at the carboxyl terminus of the dipeptide are cleaved efficiently. Differences with respect to the specificity, and activity of proteolytic enzymes from lactic acid bacteria are believed to play a central role in determining which peptides accumulate in the cheese matrix and hence whether or not a cheese will develop a bitter off-flavor.

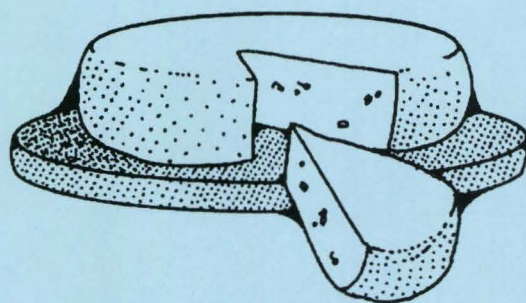
To control bitterness it is essential to control the specificity and activity of the enzymes principally involved in the formation and hydrolysis of the bitter peptides (5, 6). The formation of bitter peptides is believed to be primarily the result of the action of chymosin and the lactococcal CEP, while the hydrolysis of bitter peptides is thought to be primarily the result of the action of lactic acid bacteria peptidases. The level of chymosin in the cheese matrix is dependent on the level added and the pH at which the whey is drained. The lower the pH at draining, the greater the percentage of chymosin which will be retained in the cheese matrix. The impact of the lactococcal CEP is dependent both on its specificity and the total activity in the cheese matrix. The total activity of the lactococcal CEP is a function of the activity per cell and the final cell density reached by the starter culture. Both specificity and activity per cell can be controlled by careful selection of the starter culture. The final cell density reached by the starter culture is dependent on both culture selection and the make procedure. The activity and

specificity of peptidases from lactic acid bacteria in the cheese matrix can be controlled in a variety of ways. Starter culture selection again can play an important role. However, not only is the specificity and the activity of the peptidases important, but also the rate at which these enzymes are released into the cheese matrix. Peptidases are intracellular enzymes, therefore cell lysis is required before the enzymes will have access to peptides in the cheese matrix. Culture adjuncts can also be used to alter the level of peptidases present in the cheese matrix. A variety of lactic acid bacteria have been employed as culture adjuncts to reduce bitterness. Of the organisms examined, *Lactobacillus helveticus* has demonstrated the most promising results (1). The effectiveness of this organism is thought to be the result of its relatively high general aminopeptidase activity. Alternatively, commercially available enzyme preparations can be added to increase the level of peptidase thought to be responsible for the hydrolysis of bitter peptides.

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OFF-FLAVOR PRODUCTION IN CHEESE



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OFF-FLAVOR PRODUCTION IN CHEESE

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Flavor development in ripened cheese varieties is a complex and poorly understood process. While glycolysis and lipolysis probably contribute to flavor, enzymes involved in the hydrolysis of milk proteins are widely believed to make the most important contributions to product texture and flavor. As a consequence, many of these enzymes have been characterized and knowledge of peptides and amino acids which accumulate in ripening cheese is rapidly expanding. With the exception of bitter peptides, however, peptides and amino acids are not believed to directly influence cheese flavor, and further research on the mechanism(s) by which peptides and amino acids contribute to cheese flavor is required.

One important mechanism by which products of proteolysis may affect cheese flavor involves catabolism of amino acids by cheese microflora. The best characterized example is the production of the important cheese flavor compound methanethiol from methionine by the *Lactococcus lactis* enzyme cystathionine β -lyase. Studies of amino acid metabolism in a variety of microorganisms have shown these reactions may follow a number of enzymatic routes, each of which could potentially affect cheese flavor (Fig. 1). Catabolism of aromatic amino acids, for example, is believed to contribute to the development of off-flavors in cheese. This defect is of particular significance in reduced-fat cheeses, where the propensity for off-flavor development has contributed to reduced consumer acceptance of these products.

Aromatic compounds which are believed to produce unclean flavors in cheese include indole, skatole, *p*-cresol, and phenethanol. Metabolic pathways for the formation of some of these compounds have been described in a number of microorganisms (Fig. 2) but not in lactic acid bacteria. For this reason, it is not known whether production of these compounds in cheese occurs via enzymatic pathways, chemical reactions, or a combination of both. An improved understanding of mechanisms for the production of unclean flavors in cheese may new reveal strategies to control or even prevent this defect. For this reason, one of the objectives of the Utah State/University of Wisconsin-Madison collaborative cheese flavor project has been to investigate the catabolism of aromatic amino acids by lactococci, flavor adjunct bacteria and nonstarter lactic acid bacteria.

Figure 1. Possible avenues for microbial amino acid catabolism (adapted from Urbach 1995).

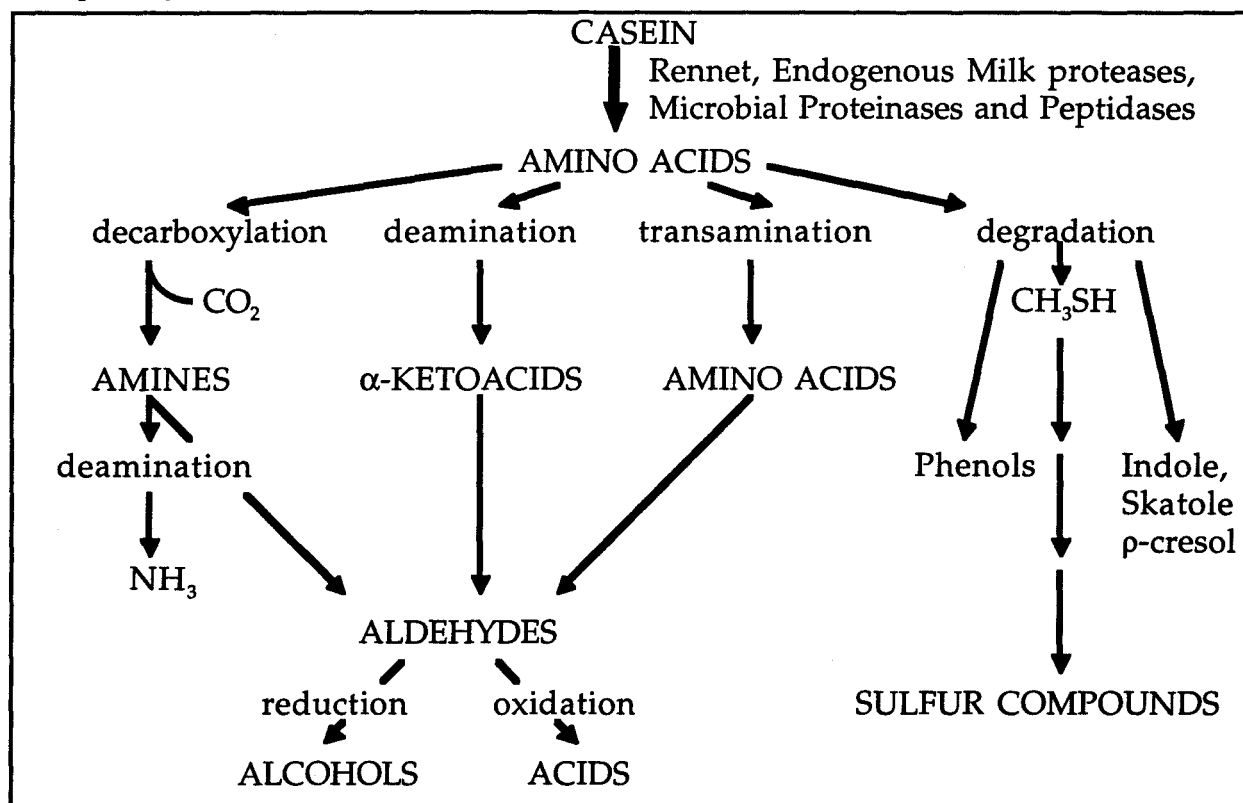
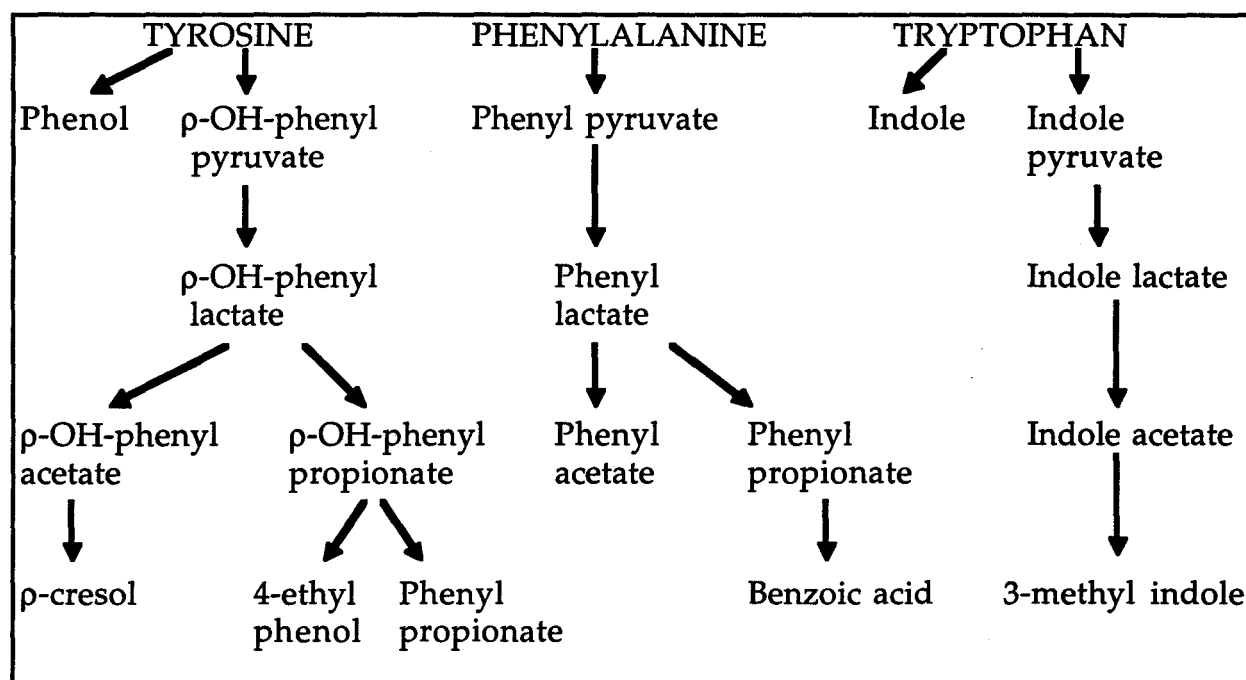


Figure 2. Production of phenolic and indolic compounds by human colonic bacteria (adapted from Macfarlane and Macfarlane 1995).



Research in Dr. Steele's laboratory at the University of Wisconsin-Madison showed catabolism of L-tryptophan (Trp) by *Lactococcus lactis* under cheese-like conditions (pH 5.2, 4% NaCl, 15°C) was initiated by aminotransferase (ATase). ATase activity was also observed with L-tyrosine (Tyr) and L-phenylalanine (Phe) in all strains examined. Histochemical staining suggested lactococcal ATase(s) had activity on L-Trp, L-Tyr, and L-Phe. The products of this enzyme(s) on L-Trp, L-Tyr, and L-Phe were indole pyruvate (IPA), *r*-OH-phenyl pyruvate (HPPA), and phenyl pyruvate (PPA), respectively.

Variation in ATase activity on aromatic amino acids among 8 strains of lactococci was insignificant, except for *L. lactis* 11007. Lower ATase activities were noted in 11007, which initially suggested this strain might not produce the levels of aromatic metabolites expected from the other strains. Under conditions that simulated those encountered in ripening Cheddar cheese, however, strain 11007 actually produced higher levels of aromatic amino acid metabolites. Those results demonstrate the potential for reaching inaccurate conclusions when results from experiments not conducted under cheese-like conditions are extrapolated to the cheese environment.

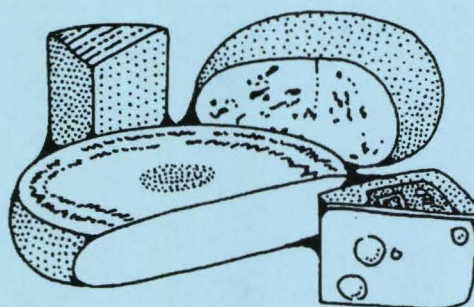
Work performed in Dr. Broadbents laboratory at Utah State indicated that catabolism of L-Tyr and L-Phe, under cheese-like conditions, by two *Lactobacillus casei* cheese flavor adjuncts was also initiated by ATase. Unlike lactococci, these bacteria were able to further catabolize HPPA to *p*-OH-phenyl lactate and *p*-OH-phenyl acetate, and PPA to phenyl lactate and phenyl acetate. Neither bacterium was able to produce *p*-cresol from Tyr or phenethanol from Phe, but capillary electrophoresis studies suggested these compounds could form spontaneously from intermediates produced by microbial catabolism of aromatic amino acids in cheese.

In summary, lack of flavor development and an increase in the occurrence of off-flavors in reduced-fat Cheddar cheese has significantly reduced consumer acceptance of these products. Previous research has indicated that the intensity of off-flavors in cheese was associated with specific cultures. This information, and the knowledge that unclean flavor compounds can be produced from aromatic amino acids, prompted us to investigate the catabolism of aromatic amino acids by bacteria found in cheese. Results of our work suggest catabolism of aromatic amino acids by lactococci and *Lactobacillus casei* is initiated by ATase, and that some compounds associated with unclean flavors can form spontaneously from microbial aromatic metabolites. Future studies will investigate possible interactions between starter cultures and adjuncts or non-starter lactic acid bacteria to more fully determine the influence of aromatic amino acid catabolism on cheese flavor.

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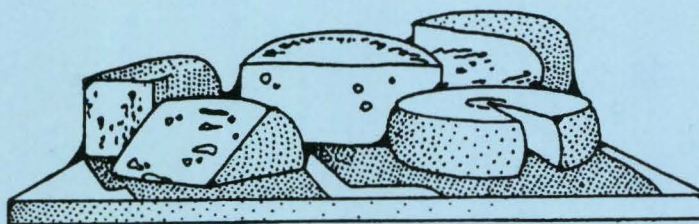
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APPLICATION OF HIGH PRESSURE IN CHEESEMAKING



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**WILL INCREASED FAT SURFACE
AREA HELP CORRECT QUALITY
PROBLEMS CAUSED BY REDUCTION
OF FAT IN CHEDDAR CHEESE**



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WILL INCREASED FAT SURFACE AREA HELP CORRECT QUALITY PROBLEMS CAUSED BY REDUCTION OF FAT IN CHEDDAR CHEESE

Presented at 12th Biennial Cheese Conference

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Introduction

We hypothesize that the milk fat interface in cheese is an important site for development of cheese flavor. Flavor development in fat-reduced cheese is limited because interface is reduced proportionally to fat reduction. Cheese milk made up of skim milk standardized with homogenized cream would provide more fat interface in the cheese. The objective of this work is to learn if homogenization of cream used to standardize skim for cheese making will improve the acceptability of fat-reduced cheese. A further objective is to investigate the effect of different surfactant proteins and protein-free surfactants in the cream.

Background and Justification

Homogenization of cheese milk has been studied and practiced but its effects on Cheddar cheese are in balance detrimental. Whey removal is impeded, curd tension is reduced, final moisture content is raised, yield is increased, curd fusion during cheddaring is inhibited and more curd dust is lost in the whey. These effects are moderated or eliminated by separately homogenizing the cream then using it to standardize the cheese milk. Cheese made from homogenized milk or separately homogenized milk is whiter due to more intense light scattering by the more numerous fat globules (Jana, 1992 a review)

Evidence is mounting that separately homogenizing cream may help correct deficiencies in fat reduced cheese. The interface may be a site of concentrated microbial activity. Laloy et.al. (1995) reported higher retention of starter in full fat than low fat cheese and observed in electron micrographs that microbial cells were concentrated at the fat/water interface. Dean et al. (1959) noted that bacteria tend to congregate at the fat-protein interface of Cheddar cheese. Lawrence (1967) and Olsen and Johnson (1990) suggested that fat serves as a reservoir for fat soluble flavors and that low fat cheese would be deficient in this respect. Olsen and Johnson (1990) along with van Vliet & Dentener-Kikkert (1982) also suggested that the interface provides a fat-water interface for reactions, and that the rheology of the cheese is affected by the contents of the fat globule membrane with casein coated globules contributing to the elasticity of the cheese and casein-free interfaces not participating in the matrix.

It may be expected that additional interface produced by homogenization could provide flavors produced in the serum more ready access to fat. Homogenization of cream in non-casein medium might also serve to soften the texture of fat-reduced cheeses.

Metzger and Mistry (1995) compared cheese made by separate homogenization of cream with unhomogenized controls and reported an improvement in the texture in the separately homogenized cheese. They reported that the hard, rubbery and curdy character of the unhomogenized controls was improved by homogenization of the cream. Most attributes were not significantly different in quality or intensity among the fat reduced cheeses. A full fat control was not included in the comparisons.

Description of experiment

Design of experiment: This experiment studies the effect of homogenizing cream on two levels of fat reduction, 1/2 and 2/3 reduction of fat in the cheese milk. It also studies the effect of different membrane materials on the homogenized fat globules. Treatments included full fat unhomogenized control (FFC), Medium fat unhomogenized control (MFC), and low fat unhomogenized control (LFC). Separately homogenized treatments included medium fat homogenized in milk serum (MFS), low fat homogenized in serum (LFS), medium fat homogenized in whey protein (MFW), low fat homogenized in whey (LFW), medium fat homogenized in polysorbate 80 (MFP), and low fat homogenized in polysorbate 80 (LFP). Each treatment was replicated.

Cheese milk preparation: All creams were at 20% fat and 43°C while being homogenized at 13.8/3.4 MPa. The milk serum cream was fortified with low heat milk powder to 20% MSFF prior to homogenization while the whey and polysorbate creams were double washed in 43°C followed by surfactant addition and homogenization. Whey cream was 10% WPC and polysorbate 80 cream was .0084% polysorbate 80. Warmed homogenized creams were used to standardize 31°C skim milk to appropriate fat levels for cheese making.

Starter: The starter was *L. lactis ssp. cremoris SI*. *Brevibacterium linens* was used as an adjunct. Both were grown up in acclimation. The adjunct was frozen as pellets in liquid nitrogen to assure uniform starter throughout the experiment. Cultures were provided by Dr. Weimer's laboratory. To prevent introduction of non-starter lactics, cheese making equipment was steamed for 20 minutes between batches and care was taken to sanitize all equipment that came in contact with the milk or cheese.

Cheese making procedures: Cheese was made in 1700 l batches. After cutting, full fat controls were warmed from 32 to 38.9°C in 30 minutes then stirred for 30 more minutes at 38.9° before draining. Curd was cheddared to a pH of about 5.6 before milling. curd was salted at 2.5% of the milled curd weight and pressed. Modifications for the low fat cheeses included warming to 37.7°C in 20 minutes followed immediately by draining. Curd was milled at pH 5.85 -5.90.

Tests conducted:

Initial analysis

1. Proximate analysis including fat by babcock, solids by CEM microwave oven, and protein by kjeldahl on milk, whey and cheese. The cheese milk was analyzed for casein.
2. Fat globule size was determined in the cheese milk by Coulter using an LS series instrument.
3. pH of cheese was determined using the quinhydrone electrode.
4. Starter, adjunct, and non-starter lactics (NSLABS) were determined in Dr. Weimer's laboratory.
5. Amino peptidase and total lipase/esterase activity was determined using colorimetric assays in Dr. Weimer's laboratory.

Analyses at 2, 4 and 6 months

1. Cheese pH by quinhydrone
2. Starter, adjunct and NSLABS were counted in the cheese
3. Amino peptidase and total lipase/esterase activity was determined in the cheese.
4. Aromatic amino acids and amino acid metabolites were determined in selected cheeses by capillary electrophoresis in Dr. Weimer's laboratory.
5. Descriptive sensory analysis of the cheese was conducted by Dr. Mina McDaniel and Sonja Rubico at OSU at 2,4 & 6 months. The panel was composed of 9 trained judges. Tillimook low fat and full fat cheese and Kraft low fat cheese were included in the set. Nine trained judges evaluated a random 4 samples per session in three sessions per day over four days. Each of the two replicates of each treatment was evaluated twice. Panelists rated each of 9 aroma attributes, 10 flavor attributes, and 9 texture attributes on a 16 point scale with 0 = none and 15 = extreme.
6. Consumer acceptance of the cheeses was determined using a 113 member consumer panel. All were users and likers of Cheddar cheese. They judged the acceptance of appearance, aroma, texture, flavor, color on a 9-point hedonic scale with 9 being like extremely and 1 being dislike extremely. They evaluated the ideality of hardness, and tartness on a 5 point just-about-right (JAR) scale with 3 being 'just about right' with higher numbers being too high and lower numbers being too low in those attributes. Each panelists evaluated both replicates of the 9 treatments plus Tillimook full fat and low fat samples. They evaluated these 20 coded samples in 5 session testing 4 samples per session. Each receive the samples in a different random order.
7. Objective texture measurements were made on the cheeses using the Stephens Farnell Texture Analyzer at USU. The Texture Profile Analysis two cycle compression procedure was used to measure hardness, cohesiveness, gumminess, chewiness, chewy index, adhesiveness, springiness, and springy index. Samples were 2.5 cm x 2.5 cm chilled cylinders. Compression was 30%.

Results

Initial analyses:

Observations during cheese making: During cheese making we observed that the curd of the unhomogenized low fat controls was noticeably firm and rough and did not spread or knit well during cheddaring. Homogenized serum and whey curds were improved but the polysorbate curd was soft and smooth with cheddaring properties like full fat cheese. The whey from the homogenized serum was noticeably clear with the polysorbate whey being more cloudy

Proximate analysis on milk, whey and cheese: In the milk, the casein/fat ratios averaged .73, 1.4, and 2.23 for full fat, mid fat, and low fat milks with some variability (Table 1). The wheys were not different in protein but the whey of the homogenized serum milks was significantly lower in fat than most other wheys. The mid fat polysorbate whey was higher than most wheys in fat (Table 2). In the cheese, as expected, the protein and moisture of the cheese were inversely related. Protein averaged about 23, 28, and 31% and moisture averaged 41, 46.5, and 47.9% for full, mid, and low fat cheeses respectively. The homogenized whey and polysorbate fat reduced cheeses were wetter than the serum and unhomogenized controls with means of 48.5 and 45.8% respectively. The fat content averaged 31.9, 19.3 and 15.8% in full, mid, and low fat cheeses respectively (Table 3).

Fat globule diameter and surface area: Fat globule volume/surface average diameter was about 4 μm for unhomogenized globules while homogenized globules were in the neighborhood of 0.8 and 0.9 μm . Square meters of surface per ml of cheese milk was about .04, .02 .07 and .11 in full fat, fat reduced unhomogenized, low fat and midfat homogenized milks respectively.

Cheese yield: Cheese yield predictably dropped from about 10 to 7.5 kg cheese per 100 kg of milk when removing 2/3 of the fat from milk. Cheese solids obtained from a kg of milk solids dropped also as the non precipitating solids remained constant in the milk while the fat declined. Cheese solids per kg of protein dropped from around 2 in full fat cheese to 1.35 in 2/3 fat reduced cheese as the protein content of the cheese increased from 23, to 29 to 31% (Table 4)

Observations at 2, 4 and 6 months:

Cheese pH: The pH of cheese was low with the full fat control and fat reduced cheeses near a pH of 5. Some of the higher moisture fat reduced cheese even dropped to around 4.9. We noted that the quinhydrone procedure gave pH's about 0.1 lower than glass electrodes. We did not observe the typical rise in pH as the cheese aged. On the contrary many of the cheeses continued to increase in acidity (Table 5).

Culture organisms in cheese: The fat reduced cheeses homogenized or not are not lower in viable starter culture cells than the full fat control. This refutes the findings of Laloy et al., 1995) in which he reported reduction of starter cells in cheese when fat is removed. Two homogenized cheeses initially had significantly higher counts than the full fat control. As the cheese aged, differences in starter counts disappeared. This data does not support the hypothesis that culture bacteria congregate on the fat interface (Table 6).

Adjunct Bacteria numbers: *Brevibacterium linens* was found only in a few of the initial cheeses but at 4 and 6 months viable adjunct organisms were found in almost all cheeses. No pattern was evident as to the effect of fat reduction or separate homogenization on the presence of adjunct organisms.

Non-Starter Lactics(NSLABS): No NSLABS were detected in the freshly made cheese. At 2 and 4 months, 2 and 3 of the 18 cheese had viable NSLABS but at 6 months, all but one of the cheeses tested has NSLAB growth. No pattern due to treatment was evident.

Amino peptidase activity: AP results were widely variable and no significant difference in AP activity between the treatments were found. Although the effect of time is not included in the analysis, it appears that the activity is high in all cheeses initially and that it decreases with time (Table 7).

Total lipase/esterase activity is not significantly different between treatments initially and at 4 and 6 months. At 2 months medium and low fat homogenized whey and polysorbate cheese are significantly low (Table 8).

Aromatic amino acids and their metabolites: The capillary electrophoretic work on aromatic amino acids and their metabolites is still being interpreted. No treatment effect is evident upon cursory examination of the data.

Objective texture measurements: At two months, hardness measurement showed that all the treatments were harder than the Tillimook products but not different than one another. All the fat reduced cheeses were more cohesive than FFC or Tillimook cheeses. Tillimook cheeses were less chewy than any of the experimental cheeses but the LFS and LFW cheeses were more chewy than the FFC. The LFP cheese was not different from the FFC in chewiness. The spring index of all the fat reduced cheeses is greater than the FFC cheese. Only LFS is more springy than LFC.

Descriptive sensory Analysis:

Texture: Focusing on the two-thirds fat reduced two month cheese, All the fat reduced cheeses are more springy than FFC and LFP is less springy than LFW; All the fat reduced cheeses are harder than the FFC and no difference in hardness due to homogenization was detected; cohesiveness was not different among all the experimental cheeses; FFC was more adhesive than all the fat reduced cheeses except LFP; FFC was more moist than all fat reduced cheeses; All fat-reduced cheeses are more curdy than FFC but LFP is less curdy than LFS. Graininess increases with fat reduction but homogenization reduces or eliminates that graininess. FFC is more grainy than LFC but LFS, LFW & LFP are not more grainy than FFC; FFC is less chewy than all the fat reduced cheeses regardless of homogenization. LFS is more chewy than LFC (Table 9).

Flavor: Among the 2/3 fat reduced cheeses at 2 months, no difference is detected in the level of sour, salt, bitter, cheesy, nutty, sulfur or yeasty/dirt flavor. The overall flavor intensity of FFC is stronger than all the homogenized cheeses but not stronger than the LFC; FFC is more buttery than LFC but not more buttery than any of the homogenized low fat cheeses (Table 10).

Aroma; The only aroma note that was different among the 2/3 fat reduced 2 month cheeses was the overall intensity. FFC had a more intense aroma than LFS and LFW (Table 11).

Consumer acceptance results:

Overall acceptability: Considering the 2/3 reduced fat treatments at 2 months, the LFC is less acceptable than FFC but the all the homogenized low fat treatments are not different in acceptability from FFC. Homogenization does improve the acceptability of the low fat cheeses. The trend holds true at the cheeses age. The MFC does not show the significant drop in acceptability compared to the FFC.

Flavor acceptability: The same trend holds in the flavor acceptance scores. Reduction in fat results in significantly less acceptable cheese but homogenization of the fat improves the flavor acceptability. Homogenized low fat cheeses are not different in flavor acceptability from FFC.

Texture acceptability: Texture acceptability follows the same trend except that only LFP is significantly more acceptable in texture than LFC.

Color acceptability: Color acceptability shows the same trend.

Hardness ideality: The hardness ideality results provide an interesting demonstration on the effect of context on the consumers concept of ideal hardness. The fat reduced treatments at 2 months all result in cheese that is too hard but FFC cheese is judged to be too soft. At 4 months the LFW and LFP are judged to be close to ideal while FFC is too soft and the LFC and LFS are too hard. This does demonstrate that the fat-reduced cheeses are harder than the FFC but that polysorbate 80 homogenized onto the membrane serves to soften the cheese (Table 12).

Conclusions:

- A. Reduction of fat in cheese milk by 50%, modification of the make procedure to hold more water, and use of appropriate cultures results in cheese that is not less acceptable than standard full fat cheese
- B. Reduction of fat in cheese milk by 67% with the above precautions results in cheese that is less acceptable in texture, flavor and color than a full fat cheese.
- C. Standardizing skim with separately homogenized cream improves the acceptability of cheese from 67% fat reduced milk so that it is not less acceptable than full fat cheese.
- E. Each of the three surfactants tested gave an increase in fat surface area and had positive effects on consumer acceptability in the cheese from 67% fat reduced milk.

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Table 1

Cheese milk composition

Treatments									
Component	FFC	MFC	MFS	MFW	MFPS80	LFC	LFS	LFW	LFPS
Total Protein	2.86 bc	2.97 ab	3.06 a	2.86 bc	2.78 c	3.09 a	3.05 a	2.90 bc	2.89 bc
Casein	2.24 bc	2.33 ab	2.40 a	2.24 bc	2.18 c	2.42 a	2.39 a	2.27 bc	2.26 bc
Fat	3.10 a	1.65 b	1.70 b	1.54 b	1.65 b	1.15 c	1.06 cd	0.91 d	1.11 c
Casein/ Fat Ratio	.73 d	1.41 c	1.41 c	1.47 c	1.32 c	2.11 b	2.28 ab	2.51 a	2.05 b
Total Solids	11.87 a	10.66 b	10.72 b	10.10 c	10.22 c	10.19 c	10.08 c	9.77 d	9.69 d

Table 2

Whey composition

Treatments									
Component	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS
protein	.81a	.84a	.84a	.81a	.79a	.85a	.83a	.82a	.79a
Fat	0.24b	0.13dc	0.06ef	0.28ab	0.32a	0.09de	0.02f	0.23b	0.16c
Total Solids	6.90a	6.69b	6.72b	6.70b	6.52c	6.67b	6.66b	6.67b	6.54c

Table 3

Cheese composition

Treatments											
Component	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS	TF	TL
Protein	23.01f	27.83de	28.81cd	26.78e	27.98de	30.67b	29.89bc	32.86a	30.96b	**	**
Moisture	41.07f	45.35de	44.03e	48.56ab	47.81abc	46.69cd	46.96bcd	49.18a	48.73ab	34.2g	44.75e
Fat	31.88b	19.88cd	20.43c	17.96ef	18.99de	16.93f	17.00f	13.95h	15.35g	36.75a	19.75dc

Table 4

Cheese yield

Treatments									
component	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS
Actual cheese yield per 100 kg of milk	10.11 a	8.53 b	8.30 b	8.13 bc	8.26 bc	7.65 de	7.82 cd	7.34 e	7.51 de
Cheese solids per milk solids	0.53 a	0.44 bc	0.47 bc	0.40 cd	0.44 bc	0.41 bcd	0.39 cd	0.36 d	0.39 cd
Cheese solids per protein	2.03 a	1.45 c	1.68 b	1.42 c	1.50 c	1.37 cd	1.42 c	1.24 d	1.39 cd

Table 5

Cheese pH

Treatments											
pH	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS	TF	TL
Month 0	4.96bcd	4.96cd	5.14 a	4.88 d	4.90 cd	5.00 b	5.02 b	4.96 bc	4.95bcd	**	**
Month 2	4.95 cd	4.94 cd	5.13 b	4.90 cd	4.89 d	4.99 c	4.99 c	4.93 cd	4.97 cd	5.19 b	5.35 a
Month 4	5.03 c	4.89 e	5.15 b	4.89e	4.89 e	4.94 de	5.00 cd	4.92 e	4.90 e	5.30 a	5.29a
Month 6	4.89 bc	4.88 bc	5.12 a	4.77 c	4.80 bc	4.91 bc	4.95 b	4.89 bc	4.87 bc	5.11 a	5.25 a

Table 6

Starter culture in cheese at 0,2,4,6, month (log 10)

Treatments									
	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS
Month 0	7.44 c	7.25 c	7.40 c	7.93 bc	6.06 bc	7.53 c	8.10 ab	8.24 a	7.92 bc
Month 2	7.89 ab	7.78 abcd	7.60 bcd	7.29 cd	7.57 bcd	7.00 d	7.85 abc	8.05 a	7.40 bcd
Month 4	7.00 a	7.07 a	7.31 a	5.83 a	6.31 a	7.06 a	6.92 a	7.08 a	6.92 a
Month 6	5.48 a	5.90 a	6.34 a	5.08 a	6.17 a	5.76 a	6.83 a	7.09 a	5.97 a

Table 7

Total amino peptidase activity in cheese during aging

Treatments									
AP	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS
Month 0	1.95 a	12.95 a	16.30 a	12.65 a	12.40 a	16.30 a	7.80 a	30.65 a	16.80 a
Month 2	0.65 a	1.00 a	2.15 a	3.90 a	2.75 a	1.70 a	0.90 a	8.00 a	1.25 a
Month 4	0.70 a	1.35 a	2.00 a	0.85 a	1.50 a	1.35 a	0.90 a	0.65 a	1.25 a
Month 6	4.70 a	5.05 a	5.75 a	4.10 a	3.60 a	5.30 a	5.35 a	4.15 a	6.65 a

Table 8

Total lipase/esterase activity in cheese at 0,2,4,6, Month

Treatments									
	FFC	MFC	MFS	MFW	MFPS	LFC	LFS	LFW	LFPS
Month 0	4.95 a	6.35 a	10.50 a	9.85 a	5.10 a	5.70 a	3.85 a	21.65 a	7.85 a
Month 2	6.10 a	6.30 a	8.45 a	1.50 c	1.55 c	5.50 ab	5.15 bc	0.05 c	0.00 c
Month 4	17.85 a	20.65 a	21.50 a	16.35 a	18.40 a	17.00 a	9.00 a	8.20 a	14.45 a
Month 6	29.10 a	41.50 a	37.50 a	18.85 a	25.35 a	37.10 a	8.85 a	29.35 a	25.95 a

Table 10 Mean and standard deviation of cheddar cheese flavor attributes*.

Attributes	Storage Time (mos.)	SAMPLES											
		Full-Fat (a)	1/2 Reduced Fat (b)	2/3 Reduced Fat (c)	1/2 Red. Fat Homo w/Milk Serum (d)	2/3 Red. Fat Homo w/Milk Serum (e)	1/2 Red. Fat Homo w/Whey Protein (f)	2/3 Red. Fat Homo w/Whey Protein (g)	1/2 Red. Fat w/Polysorbate (h)	2/3 Red. Fat w/Polysorbate (i)	Tillamook Regular Cheddar (j)	Tillamook Red. Fat Cheddar (k)	Kraft Red. Fat Cheddar (l)
Flavor-by-Mouth													
Overall Intensity	2	7.69 1.69	7.56 1.93	7.31 1.38	6.25 1.38	6.72 1.99	7.67 1.99	6.39 1.48	7.39 1.93	6.75 1.80	7.67 1.31	6.44 1.28	7.61 1.12
	4	7.88 2.01	8.00 1.98	7.53 2.54	6.81 2.02	7.50 2.14	7.84 1.95	7.16 1.61	8.06 2.17	7.34 1.88	7.97 1.62	6.88 1.52	7.94 0.98
	6	8.57 1.89	8.23 2.47	7.69 2.32	6.69 2.12	6.86 1.94	8.45 2.06	7.62 1.61	8.70 2.09	7.93 1.77	8.17 1.78	6.90 1.88	7.86 1.43
Sour	2	4.94 2.32	5.00 2.23	4.69 1.90	4.06 1.91	4.75 1.87	4.72 2.02	4.69 1.94	5.03 2.01	4.64 1.90	4.00 2.26	3.53 1.98	3.89 2.34
	4	5.00 2.31	5.47 2.06	5.69 1.82	4.63 2.04	5.03 2.27	6.31 2.25	4.94 1.98	6.31 2.07	5.25 1.76	4.84 2.30	4.63 1.76	4.47 2.09
	6	4.60 2.40	4.60 1.94	4.83 2.30	4.10 1.99	4.10 2.02	5.28 2.28	4.48 2.03	5.57 2.66	4.52 2.06	4.13 2.19	4.17 2.05	4.07 2.05
Salty	2	3.17 2.16	3.06 1.96	3.28 1.83	3.61 1.79	2.81 2.20	3.19 2.08	2.86 1.93	3.17 2.09	3.03 2.01	3.92 2.09	3.00 2.07	4.03 2.15
	4	4.41 1.85	4.31 1.12	4.06 1.66	3.88 1.38	4.00 1.76	4.53 1.39	4.00 1.46	4.09 1.96	4.03 1.40	4.47 1.63	4.03 1.49	5.09 1.99
	6	4.37 1.56	4.37 1.54	3.72 1.85	3.93 1.75	4.17 1.56	3.86 1.79	3.59 1.96	3.93 1.70	4.14 1.79	4.37 2.09	4.10 1.78	4.79 1.72
Bitter	2	2.17 2.48	2.28 2.67	2.39 2.70	1.17 1.44	1.67 1.85	2.69 2.66	2.17 2.10	2.89 2.69	2.03 2.09	2.11 1.94	2.25 2.03	2 1.96
	4	2.19 2.06	2.47 2.42	1.84 1.37	1.50 1.65	1.84 1.92	2.88 2.31	2.75 2.05	2.97 1.71	2.47 1.98	1.75 1.69	2.22 1.90	2.13 1.68
	6	3.27 2.83	3.60 2.87	3.07 1.89	1.83 1.89	1.35 1.42	4.10 2.47	2.59 2.28	3.53 2.22	2.83 2.32	1.77 2.11	1.48 1.15	2.21 1.95
Cheesy	2	3.61 1.95	3.19 1.98	2.83 1.96	3.42 1.65	3.17 1.78	3.47 2.01	3.06 1.64	3.64 1.81	2.94 1.67	5.14 1.59	2.83 1.75	5.22 1.85
	4	4.88 1.43	3.78 1.90	3.38 1.83	3.59 1.58	3.44 1.88	3.94 1.74	3.13 1.70	4.03 1.98	3.19 1.69	5.69 1.38	4.06 1.78	5.03 1.40
	6	3.47 2.15	3.60 2.16	3.10 2.30	3.90 1.97	4.45 1.80	3.35 2.27	3.66 2.50	2.83 2.71	4.10 1.93	5.53 2.19	4.62 1.92	4.28 2.30
Buttery	2	2.78 1.84	2.19 1.75	1.89 1.56	3.42 2.05	2.56 1.78	2.39 1.90	2.33 1.91	2.39 1.50	2.61 1.86	4.00 1.99	3.47 1.86	3.61 1.81
	4	2.75 1.78	1.81 1.60	1.66 1.70	3.00 1.85	2.34 1.83	2.00 1.52	2.13 1.70	1.78 1.54	1.81 1.64	3.56 2.06	3.19 1.87	3.28 2.02
	6	2.80 2.01	1.90 1.79	1.48 1.53	3.79 2.02	2.35 1.72	1.83 1.83	2.07 1.93	2.20 1.90	2.35 1.74	4.47 2.32	4.38 2.04	3.90 2.60
Nutty	2	1.19 1.77	1.28 1.91	1.06 1.43	1.39 1.79	1.44 1.73	1.22 1.59	1.31 1.55	1.25 1.42	1.25 1.54	1.92 1.93	1.89 2.00	2.47 2.47
	4	0.97 1.36	1.09 1.38	0.94 1.52	1.47 1.67	1.06 1.48	1.00 1.39	1.25 1.50	1.16 1.48	1.31 1.55	2.38 2.24	3.00 9.63	1.63 1.93
	6	1.70 2.31	1.40 1.89	0.90 1.66	1.35 1.95	1.00 1.44	0.90 1.57	0.97 1.38	1.10 2.28	1.55 2.03	2.17 2.67	1.83 1.89	1.28 1.65
Sulfur	2	1.86 1.78	1.53 2.06	1.39 1.92	0.61 1.20	0.69 0.92	2.06 1.96	1.06 1.24	1.50 1.61	1.14 1.50	1.53 2.36	0.64 1.13	1.36 1.19
	4	1.59 1.93	1.94 2.05	1.91 2.05	1.16 1.99	1.63 1.86	2.00 2.00	1.78 1.76	1.75 1.92	1.94 1.63	1.44 2.15	1.34 1.58	1.47 1.81
	6	2.77 2.43	2.43 2.64	2.35 2.26	0.86 1.60	1.76 2.25	2.38 2.19	2.14 2.39	2.27 2.24	2.28 2.43	1.90 1.88	0.76 1.35	1.00 1.73
Goaty/Dirty	2	2.14 2.40	2.75 2.32	2.36 2.23	1.19 1.56	1.47 1.89	2.28 2.33	1.47 1.59	1.83 3.09	1.72 1.89	0.64 0.93	0.81 1.19	1.22 1.57
	4	1.56 2.00	2.16 2.48	2.13 2.32	0.94 1.50	1.81 2.06	1.81 1.94	1.94 3.38	2.31 2.55	2.13 1.68	1.31 2.39	0.75 1.22	0.66 1.13
	6	1.57 1.94	2.37 2.75	2.66 2.94	0.90 1.29	1.35 2.13	2.41 2.81	1.62 2.51	1.57 2.10	1.59 2.40	0.50 1.20	0.66 1.23	1.10 1.21
Yeasty/Fruity	2	0.44 1.05	0.56 1.42	0.28 0.74	0.50 0.91	0.22 0.54	0.72 1.43	0.42 0.87	1.03 1.99	0.56 1.48	0.42 1.05	0.42 0.91	0.47 1.08
	4	0.34 0.79	0.44 0.98	0.31 0.64	0.66 1.13	0.56 0.95	0.47 0.88	0.53 0.76	0.31 0.69	0.47 0.92	0.75 1.52	0.69 1.28	0.84 1.39
	6	0.27 0.69	0.47 1.04	0.66 1.42	0.24 0.95	0.38 0.78	0.35 0.94	0.21 0.77	0.33 0.84	0.41 1.05	0.30 1.06	0.38 0.98	1.10 1.97

*Mean scores across 9 panelists x 4 replications

Table 11 Mean and standard deviation of cheddar cheese aroma attributes*.

Attributes	Storage Time (mos.)	SAMPLES											
		Full-Fat (a)	1/2 Reduced Fat (b)	2/3 Reduced Fat (c)	1/2 Red. Fat Homo w/Milk Serum (d)	2/3 Red. Fat Homo w/Milk Serum (e)	1/2 Red. Fat Homo w/Whey Protein (f)	2/3 Red. Fat Homo w/Whey Protein (g)	1/2 Red. Fat w/Polysorbate (h)	2/3 Red. Fat w/Polysorbate (i)	Tillamook Regular Cheddar (j)	Tillamook Red. Fat Cheddar (k)	Kraft Red. Fat Cheddar (l)
Overall Intensity	2	6.78 1.31	6.97 1.42	6.50 1.38	6.72 1.70	6.19 1.43	6.19 1.43	6.14 1.31	6.14 1.36	6.25 1.95	6.97 1.65	6.03 1.36	7.36 1.15
	4	6.94 1.27	7.16 1.55	6.97 1.43	6.75 1.30	7.19 1.33	6.88 1.43	6.75 1.50	6.59 1.31	6.59 1.54	7.19 1.38	6.75 1.19	7.38 1.56
	6	6.73 1.96	6.83 1.82	6.59 1.96	6.48 1.96	6.17 1.69	6.28 1.87	6.14 1.58	6.93 2.12	6.62 1.70	7.27 1.34	6.48 1.46	6.52 1.60
Buttery	2	4.22 1.93	3.75 1.84	3.94 1.85	4.17 2.12	3.83 1.99	3.92 1.68	3.89 1.80	3.33 1.74	3.78 2.11	4.17 1.89	4.17 1.94	4 2.2
	4	4.03 2.56	3.84 2.29	3.53 2.11	3.91 2.63	3.72 2.32	3.63 2.15	3.63 2.50	3.13 2.01	3.28 2.19	3.63 1.66	3.63 2.12	4.53 2.65
	6	3.27 1.60	2.83 2.02	2.41 1.80	3.83 1.73	3.38 2.13	3.10 2.16	2.93 1.79	2.57 1.89	2.69 2.00	4.20 3.19	4.45 1.92	3.83 2.39
Nutty	2	1.69 1.72	1.47 1.68	1.72 1.92	1.47 1.81	1.61 1.68	1.42 1.75	1.33 1.69	1.33 1.67	1.22 1.57	1.75 1.92	1.86 1.79	2.33 1.19
	4	1.41 1.72	1.25 1.46	1.22 1.50	1.09 1.49	1.22 1.36	1.16 1.35	1.41 1.70	1.38 1.60	1.56 1.74	1.53 1.65	1.34 1.36	2.03 1.58
	6	1.87 2.47	1.67 2.23	1.52 2.10	1.86 2.48	1.45 1.35	1.21 1.84	1.48 2.03	1.60 2.76	1.52 2.29	2.07 2.39	1.17 1.69	1.62 1.74
Sulfur	2	1.17 1.70	1.61 1.86	0.78 1.02	0.86 1.10	0.69 1.04	0.78 1.31	1.11 1.49	0.78 1.27	0.78 1.10	1.89 2.18	0.58 0.94	1.28 1.63
	4	1.50 1.95	1.34 1.86	1.69 1.82	1.13 1.72	1.44 1.70	1.53 1.72	1.34 1.72	1.38 1.68	1.56 1.72	1.41 1.95	1.31 1.58	1.59 2.00
	6	1.73 2.18	1.60 2.09	1.86 2.37	1.10 1.54	1.00 1.73	1.28 1.79	1.55 1.92	1.70 2.07	1.45 2.06	1.83 2.07	0.79 1.52	0.66 1.40
Goaty/Dirty	2	1.22 1.59	1.36 1.66	1.31 1.93	1.58 1.73	1.25 1.78	1.14 1.57	1.28 1.68	1.33 1.96	1.25 1.93	1.22 1.66	0.94 1.37	1.11 1.75
	4	1.50 1.78	1.56 2.12	1.66 1.84	1.38 1.79	1.31 1.75	1.63 1.74	1.34 1.62	1.56 1.85	1.53 1.65	1.91 2.12	1.13 1.41	1.28 1.30
	6	1.43 1.52	1.43 1.48	1.69 1.71	0.90 1.45	1.03 1.43	1.86 1.73	1.00 1.44	1.60 1.73	1.17 1.54	0.77 1.22	0.55 1.62	0.69 1.17
Yeasty/Fruity	2	0.44 0.84	0.83 1.06	0.53 1.03	0.67 1.22	0.61 0.96	0.39 0.84	0.25 0.65	0.94 1.67	0.64 1.22	0.69 1.22	0.50 0.97	0.64 1.05
	4	0.78 1.31	1.09 1.28	0.84 1.08	1.16 1.57	0.81 1.12	0.66 0.83	1.19 1.91	0.66 0.90	0.75 0.95	0.97 1.53	1.25 1.57	1.25 1.61
	6	0.40 0.89	0.47 1.01	0.79 1.21	0.10 0.41	0.62 1.08	0.41 1.09	0.45 1.12	0.80 1.22	0.90 1.15	0.17 0.46	0.38 0.73	0.93 1.53

*Mean scores across 9 panelists x 4 replications

Table 12

Consumer Sensory Panel Data at 2, 4, and 6 Months

		1	2	3	4	5	6	7	8	9	10	11
		Full Fat Control	50% Fat Reduced Control	67% Fat Reduced Control	50% Fat Reduced Hom Cream	67% Fat Reduced Hom Cream	50% Fat Reduced H. C. w/ Whey Proteins	67% Fat Reduced H. C. w/ Whey Proteins	50% Fat Reduced H. C. w/ PS80	67% Fat Reduced H. C. w/ PS80	Tillamook Full Fat	Tillamook Reduced Fat
Color	2 mo	7.107ab	6.935ab	6.048c	7.182ab	7.210a	7.201a	6.825b	6.936ab	7.275a	5.500d	5.138d
	4mo	6.961bcd	6.738cd	5.911e	7.575a	7.265ab	6.557d	6.792cd	6.663d	7.088bc	5.814e	5.300f
	6 mo	7.098	6.704	5.686	7.310	7.168	6.867	6.7566	6.805	7.075	5.400	5.150
Appearance	2 mo	7.097abc	6.741cd	6.146f	7.408a	6.983bc	7.036abc	6.724cd	6.866cd	7.242ab	6.524de	6.154ef
	4mo	6.616cd	6.367def	5.752g	7.570a	7.190ab	6.106fg	6.570ede	6.252def	6.938bc	6.495cdef	6.123efg
	6 mo	6.676	6.288	5.398	7.474	6.969	6.341	6.606	6.283	7.089	6.336	6.001
Aroma	2 mo	6.722abc	6.637abc	6.439cd	6.935a	6.740abc	6.685abc	6.542bcd	6.530bcd	6.761ab	6.306d	6.227d
	4mo	6.752ab	6.601bc	6.362c	7.044a	6.548bc	6.495bc	6.336c	6.584bc	6.438bc	6.557bc	6.548bc
	6 mo	6.932	6.712	6.478	6.845	6.491	6.394	6.522	6.531	6.531	6.761	6.150
Texture	2 mo	6.859abc	6.790abc	6.516c	6.603bc	6.680abc	6.987ab	6.655abc	6.915ab	6.955ab	7.040a	6.626bc
	4mo	6.575bcd	6.738abc	6.278d	6.946ab	6.783abc	6.455cd	6.831abc	6.544bcd	7.061a	6.805abc	6.442cd
	6 mo	6.531	6.500	6.283	6.912	6.531	6.470	6.774	6.341	6.929	6.434	6.460
Flavor	2 mo	6.768a	6.619ab	6.191c	6.789a	6.518abc	6.544abc	6.627ab	6.307bc	6.777a	6.854a	6.455abc
	4mo	6.808a	6.615ab	6.261b	6.805a	6.548ab	6.216b	6.561ab	6.433ab	6.699a	6.796a	6.460ab
	6 mo	6.770	6.562	6.319	6.615	6.518	6.266	6.531	6.323	6.633	6.566	6.257
Acid	2 mo	3.320 too high	3.338 too high	3.207 ok	2.607 too low	2.927 ok	3.342 too high	2.975 ok	3.433 too high	2.991 ok	2.677 too low	2.284 too low
	4mo	3.315 too high	3.349 too high	3.159 ok	2.460 too low	2.792 ok	3.442 too high	2.823 ok	3.491 too high	3.079 ok	2.805 ok	2.300 too low
Hardness	2 mo	2.574 too soft	3.129 ok	3.426 too hard	3.546 too hard	3.554 too hard	2.879 ok	3.33 too hard	2.902 ok	3.234 too hard	2.459 too soft	2.869 ok
	4 mo	2.448 too soft	2.982 ok	3.398 too hard	3.318 too hard	3.389 too hard	2.769 ok	3.234 ok	2.646 too soft	3.044 ok	2.212 too soft	2.513 too soft
Overall	2 mo	6.687a	6.705a	6.256bc	6.744a	6.611ab	6.669ab	6.587ab	6.445abc	6.838a	6.709a	6.138c
	4mo	6.442bcd	6.623abc	6.106d	7.004a	6.615abc	6.110d	6.584abc	6.309cd	6.854ab	6.486bcd	6.300cd
	6 mo	6.513	6.478	6.090	6.761	6.327	6.323	6.593	6.279	6.752	6.283	5.956

MANUFACTURE OF FAT-FREE MOZZARELLA CHEESE

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Presented at the 12th Bien. Cheese Ind. Conf., Aug 20-22, 1996.

Problems with Fat-free Mozzarella

1. Poor Melt
2. Difficult to shred
3. Translucent color
4. Skin formation during heating
5. Excessive browning/charring

Developmental Aims

1. Manufacture a fat-free pizza cheese that will melt and stretch when cooked in a commercial pizza oven
2. Eliminate storage time

Advantages of Direct Acidification

1. Rapid and consistent make time
2. Improved moisture retention
3. Precise pH control
4. No aging required
5. Improved economics
6. Eliminate culture expense and variability

Previous Direct Acid Method (Breene et al., JDS 47:1173, 1964)

1. Pasteurize milk at 162 F for 16 sec
2. Acidify milk at 40 F to pH 5.6
3. Set milk with rennet at 100 F
4. Cut curd and hold at 100 F for 80 min
5. Heat to 120 F in 5 min
6. Drain whey - Stretch and mold

24 h Brine for

Fat-free Direct Acid Method

1. Acidify skim milk to pH 5.7, 40 F
2. Set milk with rennet at 100 F
3. Cut curd and heal for 15 min at 100 F
4. Drain one-half the whey
5. Add GDL, stir for 25 min at 100 F
6. Drain one-half remaining whey
7. Add GDL, stir for 25 min at 100 F
8. Dry salt and stretch in 5% brine at 160 F
9. Mold and cool

Total time from cut to mold - approximately 90 minutes

Advantages of New Direct Acid Method for Fat-free Cheese

Separate renneting and drain pH
Better control of calcium release

Increase curd syneresis
Better control of cheese moisture

Reduce acid requirements

Eliminate brining

Cheese Composition

Cheese	Moisture	pH
Part Skim (Cultured)		
- Stretched	45.6%	5.30
- Pressed	39.9%	5.16
USU Fat-Free (Direct Acid)		
- Stretched	60.3%	5.41
- Pressed	59.4%	5.40
Healthy Choice	55.8%	5.31

Melt Comparison between Part Skim and Fat Free Mozzarella Cheese

Melt Distance (cm)

	<u>Part Skim</u>	<u>Fat Free</u>
Day 1	9.2	13.1
Day 7	12.9	12.5
Day 14	21.3	14.3
Day 28	13.7	13.9
Healthy Choice		7.1
Alpine Lace		7.8

Nutritional Labeling for Fat Free Mozzarella Cheese

For a 30 g serving (1/4 cup):
Calories 45 Calories from fat 0

	<u>% DV</u>
Total Fat 0g	0%
Saturated Fat 0g	0%
Cholesterol less than 5 mg	1%
Sodium 200 mg	8%
Total Carbohydrate 1g	1%
Dietary Fiber 0g	0%
Sugars 1g	
Protein 10g	

Features of Direct Acid Fat-free Mozzarella Method

1. Standard cheese-making equipment can be used
2. Manufacturing costs are similar to part-skim Mozzarella cheese
3. Make-time is shorter than PS Mozzarella
4. Ideal for fat-free process cheese
5. Can be diced immediately after manufacturing
6. Does not brown excessively
7. Melting properties can be designed to meet application needs
8. Melt test performance exceeds other fat-free cheeses
9. Refrigerated storage does not alter melting properties

Conclusions

1. Fat-free Mozzarella suitable for use on pizza
2. Excellent melt performance
3. Withstands temps of convection ovens
4. Increased opacity
5. Less susceptible to hardening
6. Can be diced immediately
7. No aging required
8. Suitable for Pasta Filata or pressed cheeses
9. Excellent for making process cheese

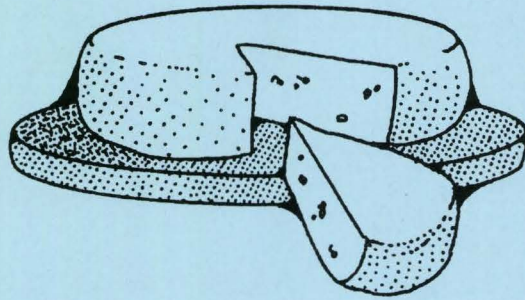
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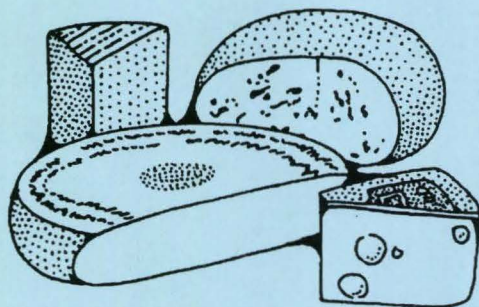
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MANUFACTURE OF FAT-FREE MOZZARELLA CHEESE



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DESIGNING CHEESES TO MEET MARKET DEMANDS



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Predicting Need for Designed Cheese

Future Consumer Trends

Designing cheese to meet the market needs must first, of course, attempt to predict those market needs. One approach is to relate future trends that are likely to occur in the cheese industry with those of the total food industry are illustrated in Table 1 (Levitt, 1993a; Sloan, 1994). Although the lists of trends were developed independently, there are several commonalities. Diet and health are major concerns, topics of discussion and focal points for advertising in the US. In a 1994 survey, 60% of the US population indicated that they have changed their eating habits to address heart disease, hypertension, cholesterol or obesity (Sloan, 1994). Over 50% agreed that cancer can be avoided with the correct actions and 44% felt that foods can be used to reduce dependency on medication.

Cheeses of fat contents ranging from skim milk to full-fat cheeses have been made in response to those demands (Levitt, 1993a). However, the markets for cheeses of lower fat contents have not evolved to the extent anticipated because of limitations in flavor and body characteristics and the necessity for higher prices. As reported in 1994, fat-reduced products accounted for less than 7% of the natural Cheddar cheese retail market, whereas light (50% fat reduction)/nonfat products made up 12% of the American (process) cheese market (Dryer, 1994). When only the volumes of light/nonfat products were compared, these types of American (process) cheese were 6-fold greater than their counterpart natural Cheddar cheese products. This reflects the advantages in formulating processed cheese to attain better flavor and texture in contrast to manufacturing natural cheese.

Table 1. Comparison of trends in the food industry versus trends in the cheese industry

Food Industry Trends	Cheese Industry Trends
Health	Light Fortified Cheese
Fresh	
Plant-based Meals	Designer Foods Beyond the Cheese Case Cross-merchandizing
Energy-enhancing Foods	
Speed of Food Preparation	Convenience On-demand Food
Eat-where-you-are Society	Shelf-stable
Microwave Magic Disappears	
Up-grading American Palate	Stronger Flavors Contrasting Textures Branded Specialities Global Cuisine Marketing to Immigrants Appetizers Desserts Micromarketing
Health-promoting bacterial cultures	Value Integrity Trend-consciousness Pourable Cheese

In the future, cheeses fortified with specific vitamins and minerals may be designed for specific markets. They will have to satisfy the requirements of the National Labeling Education Act of 1990 and compete against a variety of drinks and snack foods (Doeff, 1993). Claims can be made on labels of several existing cheese varieties that they are excellent or good sources of calcium (Alcantara, 1994). The label claim must refer the consumer to the nutrition information on the package. If specified limits for total fat, saturated fat, sodium and cholesterol are exceeded in the cheese, the label also must inform the consumer of these facts. Calcium-osteoporosis health claims can be made for cheeses that supply 20% or more of the daily calcium requirement and not more than 4g of saturated fat per serving; reduced-fat cheeses could meet these requirements. There are also some foods containing cheese as an ingredient which can claim that they are good or excellent sources of calcium. This gives the cheese industry an excellent marketing tool since cheese is usually the primary source of calcium in these foods.

Several food industry trends in Table 1 such as, fresh, energy-enhancing foods, microwave magic disappears, and use of health-promoting bacterial cultures, have no designated counterparts in the cheese industry trends. However natural cheese is commonly perceived as a fresh food. Consumer surveys indicate that "fresh" ranks first among desirable food label claims with "fat-free" a distant second. The US cheese industry may be impacted by the trend for in-home use of microwaveable foods not attaining projected sales levels. Some products such as microwaveable frozen entrees may disappear which would affect cheese sales. However, the microwave office market is growing rapidly which offers a new outlet for cheeses per se and as a food ingredient.

The perception of beneficial effects of live bacterial cultures, which has a tradition in Europe and Japan, has been accepted by American consumers. Most perceive yogurt as an exceptionally healthy food and 60% are aware of health connotations of active cultures. Special fermented milks will be the first vehicle for such cultures but cheeses may also participate in this trend. Plant-based or vegetarian-based meals are becoming increasingly popular in three major market segments: restaurants, college food service units and in-home consumption. These trends can offer significant opportunities for the combination of various cheeses with these foods.

Consumers will demand greater speed of food preparation in terms of easy incorporation of components, such as cheese, into meals prepared at home. They will also purchase more pre-prepared meals. Even though pizza is, of course, the fastest growing dinner item, greater usage of cheese in other menu items is likely to occur. Linked to rapid food preparation is the trend of Americans to become a eat-where-you-are-society. This stems from eating-on-the-run, greater number of single persons, more persons eating at work and eating in automobiles. The latter has fostered the development of hand-held luncheon items. The cheese industry has recognized these trends and realizes that high-quality, flavorful and safe shelf-stable cheeses are necessary as ingredients in these markets.

The American palate is becoming more sophisticated as indicated by the demand for more highly flavored foods and increased use of spices, greater number and amounts of ethnic dishes served in restaurants and the increase in gourmet foods and gourmet/health sections in traditional supermarkets. The number of flavored cheeses, along with mixtures of cheeses to attain contrasting textures, plus specialty cheeses have increased in response to those demands. Using cheese as an appetizer is common in the US but new types and forms of products, beyond sliced Cheddar cheese, are being introduced. There is also interest in using certain cheeses as dessert items. The industry

recognizes that this diverse use of cheese demands niche- or micro-marketing and considerable effort and capital to create a substantial market.

Marketing Trends

Marketing of cheese is segmented almost equally between retail, food service and industrial (food ingredient) channels as shown in Table 2. The profile of cheese usage has been shifting slightly as shown by changes from 1989 to 1992. The apparent inability of the retail segment to grow with

Table 2. Usage of cheese by different segments of the food industry

Industry segment	Usage in Millions of Pounds			
	1989	1990	1991	1992
TOTAL	5,893	6,211	6,368	6,697
Retail	1,978	2,026	2,150	2,071
Foodservice	2,196	2,189	2,415	2,337
Industrial	1,719	1,996	1,803	2,289

the total market in 1992 and appraisals by industry experts suggests that the only real growth in cheese sales in the near future is in foodservice or ingredient channels. In spite of this less optimistic outlook, cheese ranks among the top 25 categories of food purchases in supermarkets; 88% of supermarket shoppers purchase cheese. It accounts for 25% of total frozen/refrigerated dairy product sales. See Olson and Gould (1995) for marketing data sources.

Retail. Retail cheese marketing is very competitive and is dominated by a few major companies and private label brands which accounted for 72% of supermarket sales in 1992. During 1994, numerous new products have been introduced but the supermarket cheese display area has not grown and sales remain weak in many categories. Although shredded and grated cheeses experienced the greatest growth, their share of the retail volume in 1992 was one-half that of cheese slices and about one-fourth that of "chunk" forms (Anon, 1993). However, the percentage increase from 1988 to 1992 of the shredded/grated cheeses was 3-fold greater than cheese slices; "chunk" cheese volumes decreased during that period.

Food Service. Cheese consumption in the food service segment increased by 6.6% from 1987 to 1991 with the greatest increase being Italian varieties, primarily Mozzarella cheese (Levitt, 1993b). Cheddar cheese declined in usage. Cheese consumption in non-commercial establishments, i.e. hospitals, increased to a greater extent but 90% of the cheese is used in commercial foodservice, i.e. restaurants. In the commercial foodservice sector, restaurants and fast-food establishments are equivalent in sales and account for 90% of sales in this market. Growth was greater in the fast food sector which should be advantageous for cheese consumption. Although social caterers are a small

segment of the foodservice industry, they could also be a lucrative outlet, especially for specialty, high-value cheeses.

The foodservice industry demands competitive prices, quality, efficiency, flexibility and ideas for cheese usage. Food service operators look to the cheese industry for innovative usage of cheese in menus, especially ideas that would be exclusive for a given company. Cheese is an attractive menu item since it requires no special or sophisticated handling but some foodservice units are demanding special shapes and sizes of cheeses. Reduction in package and shipment sizes plus just-in-time delivery are becoming more common requirements. Greater efficiency and speed of food preparation is an obvious goal of fast-food chains. For example, a Mexican fast-food chain has developed a system to assemble 900 tacos per hour. Ovens used in pizza chains cook 7-inch pizzas in 40 seconds. Such systems demand close tolerances on characteristics and packaging of cheese to accommodate the inflexibility in time and steps of food preparation.

However, flexibility is essential to respond to changes in consumer trends and the specific needs of the individual foodservice companies. Demands by some operators for suppliers with multiple cheese production units to ensure a steady flow of product dictate that cheese suppliers be large. Alternatively, smaller manufacturers can share the account and cooperatively develop a standardized cheese product. Some food service companies require product consistency throughout their market whereas others want to emphasize regional cheese preferences or unique, up-scale cheeses for their customers. Mozzarella cheese has enjoyed dramatic growth during the past 10 years but may be moderating. The Mexican food market will be the largest restaurant category opportunity in the 1990's (Anon, 1993).

Industrial or Ingredient Usage. The major outlets for cheese as a food ingredient are pizza, snack foods, soups/sauces/dressings, frozen entrees and baked goods (Table 3). These account for 83% of the total usage and most have equivalent market share except for the greater usage on pizza. Of the types or forms of cheese products used as ingredients, natural cheese dominates because it captured part of the pizza market but also is used in a number of other uses shown in Table 3. Dry forms, including grated, are used in a number of foods but probably snack foods, soups/sauces/dressings and appetizers are major outlets. It is likely that snack food usage dominates since most dry products are made from Cheddar cheese. The amount of dried blue cheese is much smaller and would be used

Table 3. Categories of usage for cheese as food ingredients

Food Product	Usage as Percentage of Total Usage
Pizza	26
Snack Foods	17
Soups/Sauces/Dressings	15
Frozen Entrees	14
Baked Goods	11
Appetizers	7
Pet Food	6
Rice/Noodle Mixes	3
Shelf-stable Entrees	1

in salad dressings. Processed cheeses would be favored in uses where storage stability and controlled physical and functional (melt) characteristics are important. The large use of low fat cheeses results from the inclusion of part-skim Mozzarella cheese for pizza in this category. However, food manufacturers are demanding a greater variety of cheeses of reduced fat content or even products that can be labelled as fat-free.

Tailoring Properties of Cheese

Functional Properties of Cheese

The performance of cheese for any intended use depends, of course, on its various properties which has been termed, functionality. Functionality is not found in most dictionaries but obviously relates to the adjective functional which can be defined as "capable of performing." The term is applied to a wide variety of cheese characteristics including flavor, physical properties, stability and physiological effects. This paper will focus on physical properties and related functional characteristics. Physical properties have always been important in the traditional use of cheese as part of a meal. Brittle or short-bodied cheese can not be cut into retail sizes, especially slices, without excessive wastage. Soft, weak-bodied cheese also poses problems in cutting and in retaining its shape during distribution and marketing.

The use of cheese as a food ingredient and as an item in food service applications (restaurants) accentuated the need for specific and consistent physical properties plus the desired flavor character and intensity as shown in Table 4.

Table 4. Desired Functional Characteristics of Cheeses

- **Correct flavor intensity and flavor profile**
 - Both vary with end-use of cheese
 - **Good sliceability and shredability**
 - Under high-speed processing
 - **Prescribed softening, melting and flow**
 - These functions may be independent
 - Temperatures and times of heating vary markedly in end-uses
 - **Control of stringiness**
 - Usually limited
 - Independent of melt
 - **Independent control of flavor and physical properties**
-

Cheese must perform properly during high-speed shredding and cutting. It must soften, melt and flow at specified rates and at temperatures used during food preparation. Degree of "stringiness" of melted cheese has to be controlled. The melted cheese should exhibit the correct degree of firmness and

"rubberiness" when the food containing cheese partially cools after preparation and during consumption. These desired physical properties must be attained with the correct intensity and profile of flavors that are synergistic with the food. It is probable that flavor and physical properties will have to be regulated independently in the future which will require considerable application of science and technology.

Tailor making natural cheese for food ingredient use requires an understanding of the underlying factors which affect the properties of the cheese. This is necessary even when using various ingredients to control moisture levels or to physically disrupt the protein structure or when using added enzymes and added bacteria to alter the structure during aging of the cheese. Cheese makers are well aware of the inter-relationships between various factors and that changing one manufacturing parameter may have several unintended consequences. Figure 1 illustrates the complex inter-relationships between the various steps in cheese manufacturing and the properties of cheese during manufacturing and the finished cheese. More extensive discussion of these factors is given in reviews by Lawrence et al.(1984) and Olson (1995).

As is known by cheese manufacturers, whey is expelled rapidly from curd after cutting; this process is aided by raising the temperature of the curd-whey slurry which is being stirred in the vat. Most of the lactic acid bacteria are trapped in the curd and ferment lactose to lactic acid which diffuses from the curd. This is a dynamic system since the substrate lactose is also being removed from the curd with the expelled whey. The relationships between the rate of moisture (and lactose) removal versus rate of lactic acid production by the lactic acid bacteria, to lower the curd pH, has profound effects on the characteristics of the final cheese as shown in Fig. 1 (Lawrence, et al., 1984; Lucey and Fox, 1993). These impacts result from the rate and extent of solubilization of calcium phosphate from the protein (casein) matrix of the curd. Calcium phosphate has a substantial effect on the physical proteins of the casein aggregates. Rapid and extensive acid production will remove more calcium and phosphate, albeit less phosphate relative to calcium, to produce a brittle cheese with a lower mineral content. Several varieties of cheese illustrate the range of these inter-relationships. In manufacturing Emmental cheese, acid production is slow when most of the whey is expelled from the curd. This solubilizes less calcium phosphate and yields a cheese that is more pliable. Acid production is more rapid and extensive during whey expulsion in manufacturing Cheshire and blue cheeses which are more brittle and less firm. Other varieties can be positioned between these extremes.

Moisture and pH Control

Physical properties of cheese are also influenced by the pH of the cheese which dictates the state of the calcium-phosphate-casein structure. The minimum pH of cheeses is usually reached within the first few days of maturation. It is regulated by the amount of lactose fermented to lactic acid and the buffering capacity of the curd during manufacturing and of the cheese (Figure 1). Buffering capacity is determined by concentrations of undissolved calcium phosphate, caseins and lactate remaining in the cheese (Lucey and Fox, 1993). Acid produced during early stages of cheese manufacturing will not be buffered as extensively because of higher moisture content of the curd. Acid produced later during manufacturing will be buffered to a greater extent with the higher concentration of buffering constituents. The pH of curd during whey expulsion also affects the degree of retention of the milk-clotting enzyme, chymosin, as shown in Fig. 1; lower pH values cause

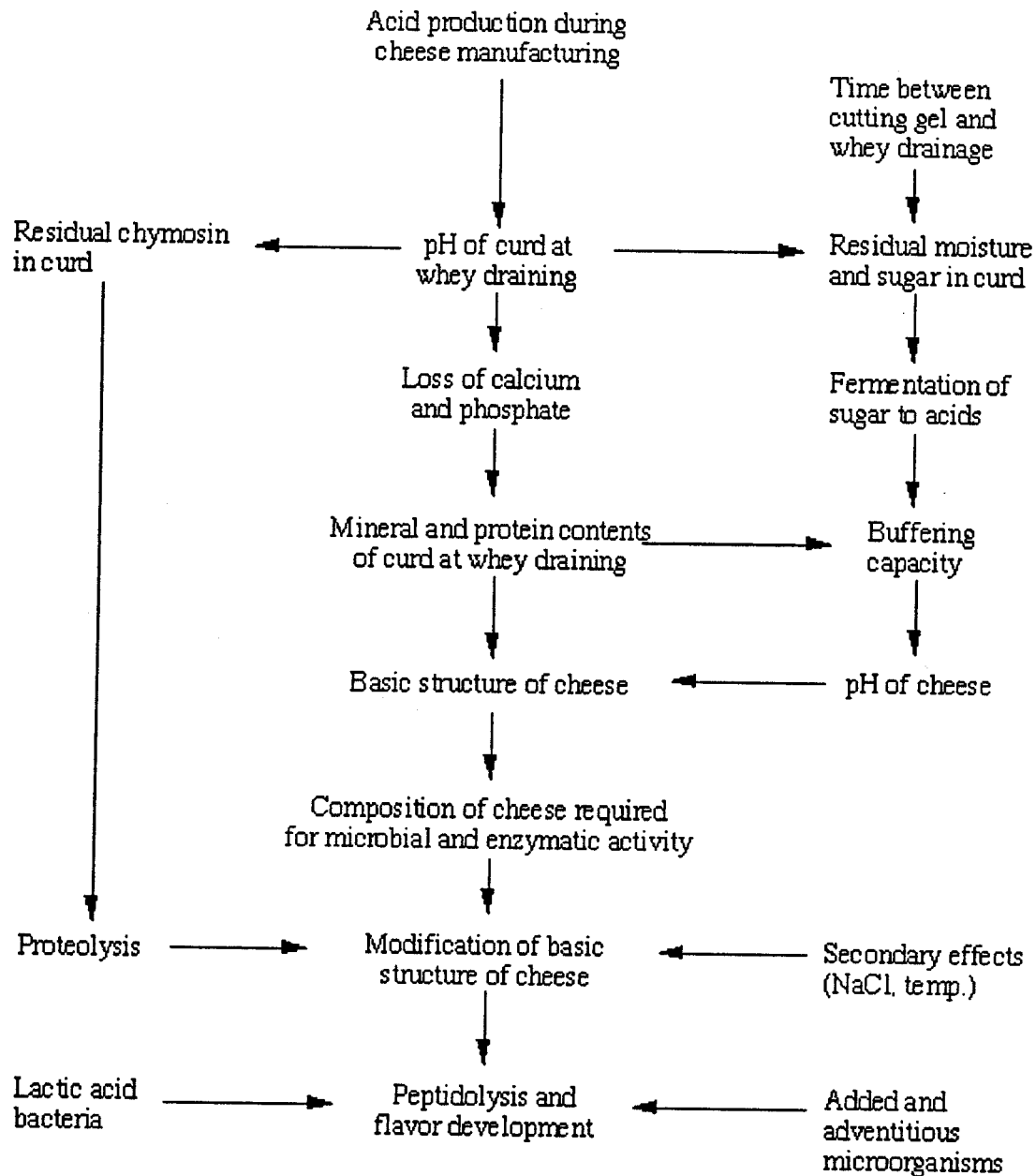


Fig. 1. Interrelationships between lactose fermentation, acid production and whey expulsion and the calcium retention, structure of cheese and proteolysis during maturation.

greater retention. This will accentuate the impact of low pH, which depletes calcium phosphate from the cheese matrix, by enhanced proteolysis of that structure to create a weaker and more brittle cheese such as Cheshire. The opposite effects occur in cheeses such as Swiss and grana-type Italian cheeses in which pH values are higher and higher temperatures are used in manufacturing which will partially inactivate rennet extract (calf rennet or fermentation-derived chymosin). Retention of other milk-clotting enzymes is not affected similarly by pH.

Curd Handling

When the appropriate moisture and pH levels have been attained for a particular type of cheese curd, the free whey is completely removed from the curd. Physical properties and pH of the curd at this stage affect curd fusion and appearance of the finished cheese. Fusion of Cheddar cheese curd does not occur until a pH of 5.8 is reached (Lucey and Fox, 1993). Presumably, this applies to other varieties unless higher temperatures and higher moisture contents permits fusion at slightly higher pH values. Removal of whey from curd before fusion yields cheese with numerous openings; fusion in the presence of whey produces a dense-bodied cheese. A dramatic decrease in moisture content of the curd occurs with any curd fusion system because of the external pressure applied to the curd (Olson, 1995).

Mozzarella Cheese

Since Mozzarella cheese is the most widely used variety as a food ingredient, it is not surprising that this variety has received most attention in terms of assessing physical properties important in food ingredient usage. Several reviews have focused on basic and applied aspects of this area (Kindstedt, 1990, 1991, 1992, 1993; McMahon et al., 1993).

Yun et. al. (1993a-d) recently assessed the effects of pH at milling and type of milk-clotting enzyme on the composition and functional properties of Mozzarella cheese. Milling cheese curd at 5.1, 5.25 or 5.4 did not affect texture profile analysis (cohesiveness, hardness, springiness) nor meltability. The lack of effect on cohesiveness may have resulted from the finished cheeses having pH values of 5.1 to 5.3 which fall in a range that would impart greater extensibility to the cheese. Since manufacturing conditions were regulated to produce similar moisture contents and fat-in-dry-matter (FDM), hardness of cheeses was similar. Meltability of cheese disks at 100°C did not correlate with pH at milling but apparent viscosity of melted cheese increased as cheese pH increased. Using *Cryphonectria (Endothia) parasitica* as a milk-clotting enzyme caused greater hydrolysis of casein and enhanced meltability but hydrolysis of α_{s1} -casein was similar to levels in control cheeses. The same effects were observed with Cheddar cheese as described later in this paper.

Softening and melt of Mozzarella cheese are enhanced with increases in moisture and fat contents (Kindstedt, 1993; Tunick et al., 1991). The apparent viscosity of commercial Mozzarella cheese samples was inversely related to moisture content. Similar relationships were observed between moisture level and disk-meltability values.

Cheddar Cheese

The increased diversity of natural cheese usage as a food ingredient prompted a large-scale study at the University of Wisconsin-Madison. The effects of milk composition, cheese manufacturing procedures and cheese composition on physical, functional and sensory characteristics of Cheddar-type cheese were assessed during 270 days of storage at 7°C. The experimental variables and analytical methods are shown in Table 5. The experimental variables were chosen to encompass changes which could be made in the composition of milk and the manufacturing of cheese on a commercial scale. The properties measured included important chemical and physical properties but meltability of cheese will be emphasized in this paper. Thermal meltability was measured by an adaptation of the Olson and Price method (1958). A 15.0±0.1g cylinder (30mm diameter X 20mm

height) of cheese was placed in a glass tube that was held horizontally in a water bath at 94°C. The flow of cheese from the leading edge was measured in millimeters at 5, 8, 12 and 15 minutes. Microwave meltability will be described later in the paper.

Multiple Regression Analysis. The large number of experiments and experimental variables necessitated analysis of the data by statistical analysis. Measurements of flow after 5 minutes of heating were consolidated for all experiments and subjected to Multiple Regression Analysis to ascertain the effects of selected compositional variables on meltability. Analyses for data at 15 and 45 days of aging are shown in Table 6. There were 181 observations in the 15 day analysis and 165 at 45 days. The coefficients of determination, adjusted for degrees of freedom, for the regression analyses were .67 and .52. The variables shown in Table 6 were selected on the basis of their potential to affect physical properties of cheese. They illustrate varying degrees of impact on meltability and would not all be included in an optimized multiple regression equation to describe meltability. The data on meltability and other functional characteristics are being evaluated using Stepwise Regression Analysis, Principal Component Analysis and Neural Network techniques.

Of the six compositional factors included in the analysis at 15 days, all except FDM and per cent hydrolysis of α_{s1} -casein had statistically significant effects ($p \leq 0.05$) on meltability. However, it should not be concluded that FDM did not affect meltability. The lack of effect of FDM resulted from a non-linear, biphasic relationship between FDM and meltability. Variations in FDM between 20 to 45% had no effect on meltability but meltability increased significantly as FDM increased from 48 to 56%. To account for this relationship, the variable SEGFDM defines the biphasic relationship in which variations in FDM between 28 and 45% had no significant effect on meltability but increases in FDM above 45%

Table 5. Experimental variables used and attributes or properties of Cheddar cheese evaluated in the Wisconsin study.

Experimental Variables:
(evaluated singly or in combination)

- Casein: fat ratio of milk
- Incorporation of low melting fat fractions
- Calcium chloride addition to milk
- Milk pasteurization temperature
- Ultrafiltration of milk
- Milk acidification (level and type of acid)
- Rennet extract (level and type)
- pH values reached during manufacture
- Starter culture proteolytic activity
- Citrate level and use of homofermentative vs. heterofermentative cultures
- Heat treatment of curd during manufacture

Attribute/properties measured:

Physical

- uniaxial compression (hardness, brittleness, adhesiveness, cohesiveness)
- thermal meltability
- microwave meltability
- sliceability

Chemical

- gross composition (moisture, fat, salt)
- cheese pH
- 12% TCA soluble nitrogen (extent of proteolysis)
- urea-polyacrylamide gel electrophoresis
- calcium/mineral analysis

Sensory

- Cheddar flavor, acid, bitter, off flavour intensity, firmness, smoothness, flavour preference and texture preference

caused increased meltability. The parameter estimate for SEGFDM indicates that a 1% increase in FDM, above 45% FDM, would increase melt by 2.5 mm. This would be equivalent to almost a 10% increase in meltability since the mean meltability was 26.7mm for the samples included in Table 6. It should be emphasized that the parameter estimates have not been normalized so care must be taken in applying them in estimating numerical effects on meltability.

The statistical significance and correlation (+ or - parameter estimate) for the variables at 15 and 45 days generally agree with expected effects. Moisture-in-the-nonfat-portion (MNFP) was a statistically significant variable and had a positive impact on meltability at both ages. It was a significant variable throughout aging and was the only significant variable of seven at 270 days of age. However, the correlation between MNFP and meltability was low at each age because the relationship was especially weak for cheeses with FDM >45%. Cheese containing <45% FDM exhibited stronger correlations between MNFP and meltability after 45 days of aging. Cheese pH was inversely related to meltability through 90 days of aging but was not a significant factor at 180 and 270 days. Total calcium concentration was a significant variable and was inversely related to meltability at 15 days but was not significant thereafter. The effect of cheese pH and concomitant increased solubility of the calcium phosphate in cheese undoubtedly had a greater effect on the structural integrity of the cheese and meltability. These results should be treated with some caution since the relationships, as expressed by Coefficient of Determination (r^2), were weak at 45 days and thereafter during aging.

The percentages of hydrolysis of α_{s1} - and β -caseins were measured only at 15 and 45 days. Meltability was not significantly ($p \leq 0.05$) influenced by the degree of α_{s1} -casein hydrolysis but β -casein hydrolysis had a significant and positive relationship with meltability at both ages. This effect resulted, to a great extent, from experiments in which *Cryphonectria parasitica* milk-clotting enzyme was used which

Table 6. Multiple regression analysis of thermal meltability (5 minute heating time) showing significance of selected compositional variables on meltability.

<u>15 days of age</u>		
<u>Variable</u>	<u>Parameter Estimate</u>	<u>Prob> T ^a</u>
Intercept	87.7	0.0076
SegFDM	2.5	0.0001
Cheese pH	-21.7	0.0001
MNFP	0.99	0.0105
FDM	0.01	0.9663
Calcium	-8.2	0.0002
α_{s1} -cas	-0.07	0.0623
β -cas	0.22	0.0006
<u>45 days of age</u>		
<u>Variable</u>	<u>Parameter Estimate</u>	<u>Prob> T ^a</u>
Intercept	47.0	0.2208
SegFDM	1.78	0.0007
Cheese pH	-16.7	0.0013
MNFP	0.96	0.0216
FDM	0.00	0.9950
Calcium	1.03	0.7407
α_{s1} -cas	-0.06	0.1081
β-cas	0.23	0.0001
TCA sol N	0.02	0.3027

^a Significant relationships shown in bold.

caused greater hydrolysis of β -casein. The level of TCA-soluble nitrogen was not a significant variable throughout aging.

Cheese Fat Level. Cheese of reduced fat content were much less meltable during early stages of aging as compared to cheese with normal fat content. However the meltable (5 minute heating) of reduced fat cheeses increased much more rapidly during aging and was similar to that of control cheese at 180 and 270 days of age (Fig 2). Two levels of fat reduction, 33 and 50%, were averaged in the reduced-fat cheese melt data since meltable did not differ significantly between these two types. The reasons for the difference in trends of meltable during aging between the reduced fat cheeses and those of normal fat content are not apparent from cheese compositional data. The MNFP did not differ significantly between control and reduced-fat cheeses. Proteolysis was not a factor since percentages

of hydrolysis of α_{s1} -casein were higher in the controls as compared to the reduced-fat cheeses and there were no differences in β -casein hydrolysis. As indicated earlier, the latter was shown to have a greater impact on meltable of cheese, as observed in the experiment in which *Cryphonectria parasitica* was the milk-clotting enzyme. Levels of TCA-soluble N were not significantly different during aging.

The pH values of cheese in which fat content was reduced by 33% were about 0.06 units higher than the controls whose mean pH values ranged between 5.06 to 5.13 during aging. The pH values of 50% fat-reduced cheeses were 0.09 to 0.19 units higher than the control during aging. Possibly the greater mobility of the protein in the higher pH cheeses enhanced the effects of proteolysis during aging, even though the levels of proteolysis in reduced-fat cheeses were less or equivalent to the controls. Although the ratio of water to protein was similar in the cheeses, the greater volume of water in reduced-fat cheeses could facilitate temperature-induced mobility of partially hydrolyzed cheese protein as the cheese ages.

Cheese pH. The impact of cheese pH on trends in meltable during aging is illustrated in Figure 3. Cheddar cheeses of normal fat content were made with lactococcal strains exhibiting low and normal proteolytic activities which were designated as Prt^d (proteinase deficient) and Prt⁺ (proteinase positive). The mean pH values during aging for cheese made with the Prt⁺ strains were 0.3 to 0.4 units lower than that made with the Prt^d strains. The unexpected drop in meltable from 15 to 45 days for the cheeses made with the Prt⁺ strains appears to associated with cheese pH. In two of three replicate trials of these cheeses, pH values at 15 days were 4.95 to 5.09. Meltables decreased at 45 days in all eight lots of cheese in both of these trials. Four lots of cheese in the third

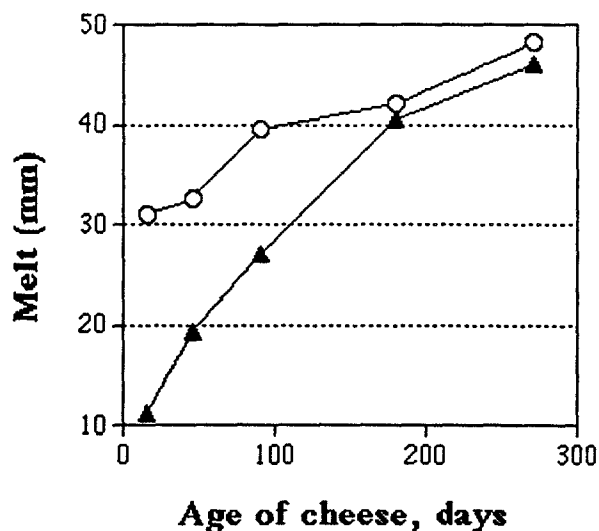


Fig. 2. Meltable (5 min heating period) of full-fat (O) and reduced-fat (▲) Cheddar cheeses during aging.

trial had pH values between 5.2 and 5.3 and exhibited an increase in meltability between 15 and 45 days. Similarly, all cheeses made with Prt^d strains had pH values between 5.22 and 5.63 at 15 days and meltabilities increased between 15 and 45 days for nine lots, remained constant for two lots and decreased in one lot. Meltabilities increased in all trials after 45 days but the rate of increase was slightly greater for cheeses made with the Prt^d strains.

Different trends were observed in a second experiment in which the two different strains were used to manufacture Cheddar cheese in which fat contents were reduced by 33 or 50% (Fig 4). The drop in meltability between 15 and 45 days was not observed in contrast to the previous experiment. Meltabilities of cheeses made with Prt^+ strains were significantly greater through 90 days of aging than those of cheeses made with Prt^d strains but the significance disappeared at 180 days and at 270 days. These trends were caused by the slightly faster increase in meltability of cheeses made with the Prt^d strains during aging. Oberg et al. (JDS 291) also observed greater meltability during aging of Mozzarella cheese made with Prt^+ strains of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus salivarius ssp. thermophilus* as compared to cheese made with Prt^d strains. However, the differences in meltability were virtually the same at all ages which discounts effects of differing proteolytic activities of the starters. The pH of cheese is a more likely factor since the pH values of cheeses made with the Prt^d strains were higher during aging.

Cheese Proteolysis. The previous discussion and multiple regression analysis suggests that proteolytic activity of cultures had little influence on thermal meltability of Cheddar cheese. Hydrolysis of β -casein

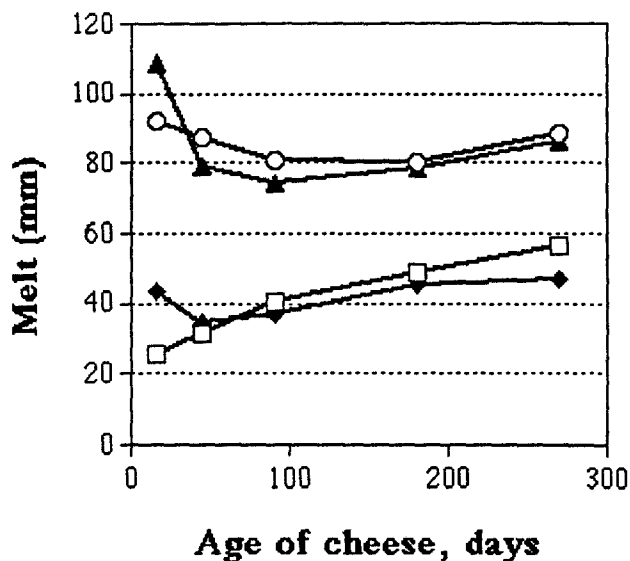


Fig. 3. Thermal Meltabilities, during 5 (\square, \blacklozenge) and 12 min (\circ, \blacktriangle) heating periods, of Cheddar cheeses made with proteinase-positive (\circ, \blacklozenge) and proteinase-deficient (\blacktriangle, \square) lactococci.

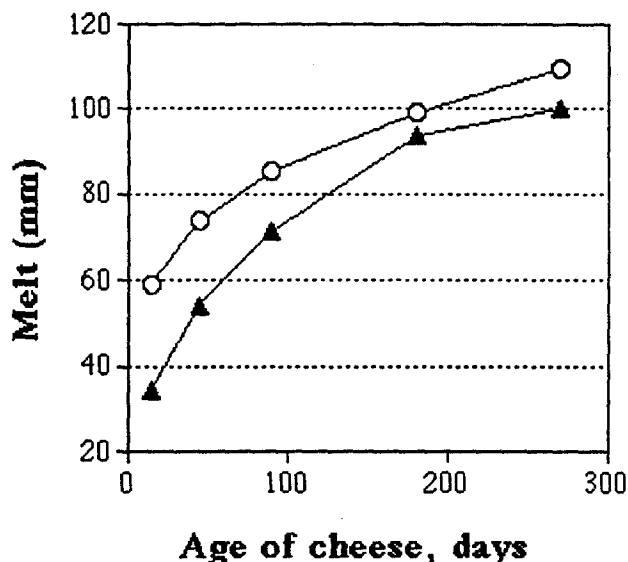


Fig. 4. Meltability (12 min heating period) of reduced-fat Cheddar cheeses made with proteinase positive (\circ) and proteinase deficient (\blacktriangle) lactococci.

appeared to be associated with degree of meltability. In two experiments in which levels of calf rennet were reduced by one-half, cheese meltabilities were not significantly influenced over the entire aging period. Hydrolysis of α_{s1} -casein, measured at 45 days, was significantly lower when rennet levels were reduced but effects on β -casein hydrolysis were negligible. In another experiment, in which calf rennet and *Cryphonectria parasitica* milk-clotting enzyme were compared, cheese meltability was significantly enhanced at all ages by the microbial enzyme. This effect was associated with the level of β -casein hydrolysis; 34 and 60% hydrolysis were observed at 15 and 45 days when the microbial milk-clotting enzyme was used. Typical hydrolysis levels in cheese made with calf rennet extract were approximately 8 and 16% at these ages. No significant differences were observed in hydrolysis of α_{s1} -casein. The meltabilities of cheeses made with the microbial enzyme also increased more rapidly during aging as compared to cheeses made with calf rennet as indicated by the highly significant aging- time:milk-clotting-enzyme-type interaction.

Another index of proteolysis, TCA-soluble nitrogen, also did not relate closely to thermal meltability. Cheeses made with Prt⁺ lactococcal strains contained significantly higher levels of TCA-soluble N throughout aging. The rate of increase in TCA-soluble N was greater also during aging as compared to cheeses made with Prt^d strains. However, differences in meltability between these two types of cheese diminished as aging progressed. Adjusting calf rennet levels slightly increased TCA-soluble N at later stages of aging. However, these differences did not significantly affect meltability. Also, there were no relationships between levels of TCA-soluble N and meltability of cheeses made from milks given different heat treatments as described below. The only experiment in which levels of TCA-soluble N correlated with meltability was in the comparison of *Cryphonectria parasitica* enzyme and calf rennet. However, differences in the TCA-soluble N levels in this comparison probably reflected the greater hydrolysis of β -casein and its impact on meltability.

Heat Treatment and Ultrafiltration of Milk. Cheeses made from milk pasteurized at 78 or 82°C were significantly less meltable at 15 days as compared to cheese made from milk pasteurized at 73°C but the higher heat treatments had no significant effect during subsequent aging. Pasteurization of milk at 85°C reduced cheese meltability throughout aging as compared to pasteurization at 73°C but the effect of the higher heat treatment was much more dramatic if milk was ultrafiltered by 2X after heat treatment. Pasteurization of milk at 73°C before ultrafiltration (2X volume reduction) produced a less meltable cheese at 15 days of age compared to pasteurization of regular milk at that temperature. However, meltabilities were the same during subsequent aging.

Microwave Meltability

Cylinders of cheese, of same dimensions as the thermal melt test, were placed in a microwave oven and increase in diameter was measured after 45 seconds. The microwave oven was a Sharp Carousel II. The multiple regression analysis shown in Table 3 described the trends in meltability more closely than the analysis for thermal meltability. The coefficient of determinations for the microwave meltability equations were 0.80 and 0.81 for cheeses at 15 and 45 days. The coefficients only decreased to 0.67 over the remaining aging period.

Several factors, SEGFDM, cheese pH, total calcium levels, and degree of β -casein hydrolysis, which influenced thermal meltability, also were significantly related to microwave meltability at 15 days of age. FDM levels were significantly related to meltability indicating that FDM levels between 28 and 45% influenced melt, albeit less than the effect above 45%. In contrast to thermal meltability,

MNFP was not significantly related to microwave meltability. All variables retained their significant relationships at 45 days except total calcium concentration. During subsequent aging, only cheese pH and FDM remained as significant variables. However, the correlation between pH and meltability was weaker than that between FDM and meltability. A physical property, cohesiveness, of cheese became one of the most closely correlated (negative correlation) factors with microwave meltability as the cheese aged. Cohesiveness is the ability of cheese to retain its structure after compression and therefore should be related to factors such as effects of cheese pH on the cheese protein matrix. The loss of significance for SEGFDM indicates that the relationship between FDM and meltability became more linear as the cheese aged. Level of TCA-soluble N became a significant factor at 180 and 270 days.

Cheese Fat Level. In contrast to thermal meltability, the rate of increase in microwave meltability of reduced fat cheeses was not substantially greater during aging than the increase in meltability of cheeses of normal fat content (Fig 5). This may relate to the lesser effect of MNFP levels and the greater impact of FDM on microwave meltability.

Cheese Proteolysis and pH. Cheeses made with *Cryphonectria parasitica* were also more meltable at all ages compared to those made with calf rennet. However, the differences in meltability between these two types of cheeses did not become greater with aging as occurred with thermal meltability. This may also relate to the greater influence of FDM relative to MNFP on microwave meltability. The effects of proteolytic activities of the starter culture on microwave meltability were similar to that observed with thermal meltability. Cheeses of normal or reduced fat contents and made with Prt^d strains were less meltable than those made with Prt⁺ strains but the difference lessened as the

Table 3. Multiple regression analysis of microwave meltability showing significance of selected compositional variables on meltability.

<u>15 days of age</u>		
<u>Variable</u>	<u>Parameter Estimate</u>	<u>Prob> T ^a</u>
Intercept	156.5	0.0001
SegFDM	1.3	0.0001
Cheese pH	-21.4	0.0001
MNFP	0.2	0.5698
FDM	0.46	0.0001
Calcium	-4.3	0.0042
α_{s1} -cas	-0.03	0.3212
β-cas	0.16	0.0003
<u>45 days of age</u>		
<u>Variable</u>	<u>Parameter Estimate</u>	<u>Prob> T ^a</u>
Intercept	207.5	0.0001
SegFDM	1.0	0.0006
Cheese pH	-31.6	0.0001
MNFP	0.19	0.4030
FDM	0.47	0.0001
Calcium	-1.7	0.3297
α_{s1} -cas	-0.02	0.2123
β-cas	0.16	0.0001

^a Significant relationships shown in bold.

cheeses aged. This also implies that the difference in pH values of the two types of cheeses was the dominant factor as in thermal meltability. Differences in proteolytic activities of lactic starter cultures did not significantly influence meltability.

Pasteurization Temperature and Ultrafiltration. Pasteurization of milk at 78 or 82°C versus 73°C did not affect microwave meltability significantly. Variability in meltability in this experiment appeared to be related to other factors such as pH, brittleness and cohesiveness of cheese. Pasteurization at 85°C yielded cheese that was significantly less meltable at all ages compared to pasteurization at 73°C. Pasteurization of milk at 85°C before ultrafiltration produced the least meltable cheese at all ages. Meltability of cheeses increased at about the same rate during aging regardless of treatment.

Rheological Properties

Properties of cheese were measured by Texture Profile Analysis, using 20% compression, and by force-deformation to 80% compression. Cylinders of cheese were compressed at 50.8mm min⁻¹. Forces (stresses) required to compress the cheese 20% and 80% of original height and also the force and amount of compression (strain) at the point of fracture were used in data analysis. The strain at fracture was converted to true strain and can be viewed as a measure of cheese brittleness.

The relationship between firmness (stress), at different degrees of compression of cheese, and certain properties of cheese can be complex. Cheeses varying in fat content exhibited unique patterns of firmness during aging as shown in Figure 6. Firmness or stress at 80% compression differed significantly during early aging periods with firmness increasing with reduction in fat. However, the rate of decrease in firmness was greater as the cheese fat content was reduced. Patterns of firmness at 20% compression during aging were also unique. Stresses at this lower degree of cheese deformation increased during aging of cheese with normal FDM. Cheeses in which fat content was reduced by 33% exhibited a decrease in firmness during the first 90 days and then an increase. The decrease in firmness of 50% fat-reduced cheeses was greatest and persisted through 180 days before a slight increase occurred.

The effects observed in Figure 6 probably resulted from effects of cheese pH values to a greater extent than effects of fat levels. The pH of full-fat cheeses approximated 5.0 whereas mean pH values of reduced fat cheeses ranged from 5.2 to 5.4. The protein in cheeses having pH values above 5.2 is more rigid because of greater bonding through hydrophobic associations, salt bridges and amorphous calcium phosphate (van Vliet and Walstra, 1994). As the pH decreases to 5.2, calcium phosphate is almost totally solubilized and the bonding between cheese proteins appears to

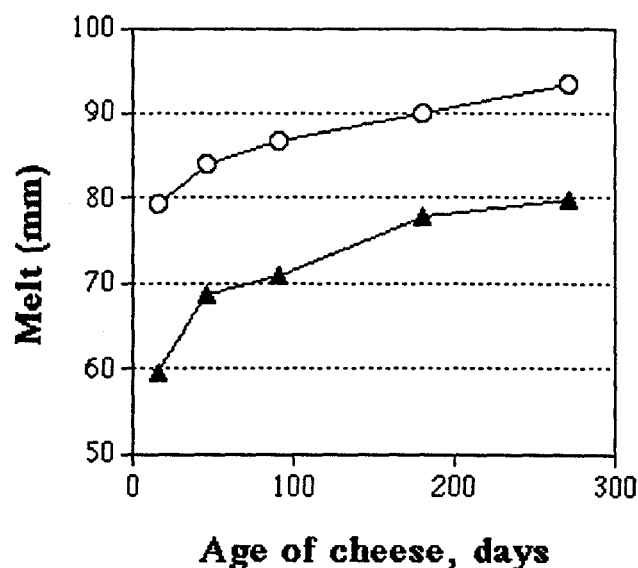


Fig. 5. Microwave meltability of full-fat (O) and reduced-fat (▲) Cheddar cheese during aging.

be weakest. At lower pH values, increased electrostatic interactions maintain the protein structure but allow only slow rearrangements as compared to pH 5.2. Hydrolysis of the more-highly structured protein in the reduced-fat cheeses appeared to have a greater impact on the integrity of this structure when compressed by 80% as compared to the more "brittle" protein structure in the full-fat cheese which had a lower pH. It is also likely that the greater volume of protein per unit mass of the reduced fat cheese was involved in the relative rates of firmness changes during aging. The increase in firmness at 20% compression (Figure 6) of the lower pH full-fat cheese during aging probably results from increased viscosity of the serum phase and uptake of water caused by proteolysis during aging. Increases in compression modulus (stress at low strains) during aging have also been reported for Gouda cheese (Visser, 1991). Multiple regression analysis indicated that stress at 80% compression was positively correlated with extent of hydrolysis of α_{s1} -casein but not with hydrolysis of β -casein.

Conclusions and Future Research

The physical properties of cheese varieties are being described and defined to a greater extent as more fundamental research is related to the chemical characteristics of those varieties. However, several areas will need to be addressed if functional properties of cheese are to be measured and used to control the properties of cheese. Better tests for melt/flow and rheological properties must be developed that are convenient, sensitive and can be related to fundamental physical properties and to conditions of using cheese in different applications. Relating results of instrumental methods to applications is especially critical since suppliers of cheese presently feel that they must evaluate cheese properties under conditions of end-use, i.e. melting in toasted cheese sandwiches. This is satisfactory for short-term product development and modification but will not give the cheese industry an essential understanding of factors that control physical

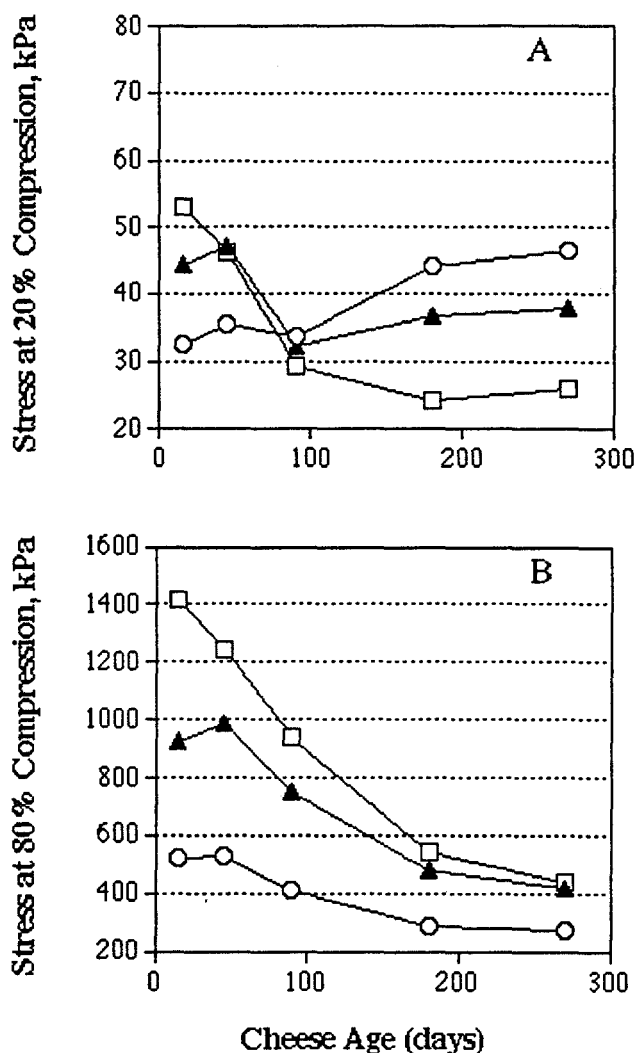


Fig. 6. Changes in stresses at (A) 20% and (B) 80% compression of full-fat (○), 33% reduced-fat (▲) and 50% reduced-fat (□) Cheddar cheeses during aging.

properties of cheese. Such an understanding will be especially important with the rapidly changing systems of food preparation which will demand close tolerances for cheese characteristics and will impose more severe conditions during food preparation.

Physical characteristics of reduced-fat cheeses also have to be defined and techniques to modify the structure of these cheeses are needed to mimic properties of cheeses of normal fat contents. Relating physico-chemical properties to rheological characteristics appears to be the first priority. These relationships can then be applied to modify the structure of reduced-fat cheeses under conditions of end-usage.

Acknowledgements

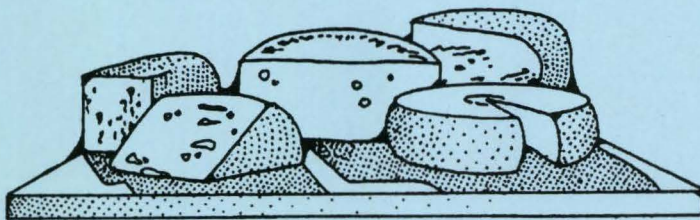
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PERFORMANCE OF LOW-FAT CHEESES IN FOODS



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Performance of Low-fat Cheeses in Foods

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Utah State University

Category 1. Cheese melted and blended with other ingredients

- cheese sauce *
- Welsh rarebit
- cheese soup
- cheese soufflé
- cheese fondue'

Category 2. Cheese melted in a product but not blended with other ingredients.

- grilled cheese sandwich *
- quiche *
- microwaved cheese sandwich
- baked macaroni and cheese

Category 3. Food incorporated into nonliquid system and heated.

- cheese bread*
- cheese sticks or cheese crackers

Category 4. Cheese melted on top of food system.

- scalloped potatoes
- potatoes au gratin *
- enchiladas
- casseroles

Category 5. Cheese blended with other ingredients but not heated.

- cheese ball*
- cheese salads/marinated cheese

Samples

- Cheeses:
 - Full-fat
 - Reduced-fat
 - No-fat
- Two replications of cheese samples
- Testing at 2, 4 and 6 months

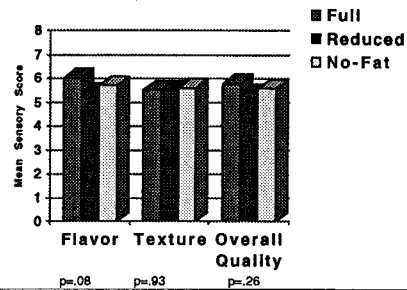
Cheese Composition

	Full	Reduced	No-fat
Fat	34±1	20±1	1.1 ±.2
Protein	16±2	22.1±.2	27±1
Moisture	38±2	47±1	55± 1

Sensory Methodology

- Open panels
- Cheese sauce, grilled cheese, quiche, potatoes au gratin, cheese bread, and cheese balls
- Products served at their typical service temperatures
- Flavor, texture, and overall quality evaluated using 9-point hedonic scale.

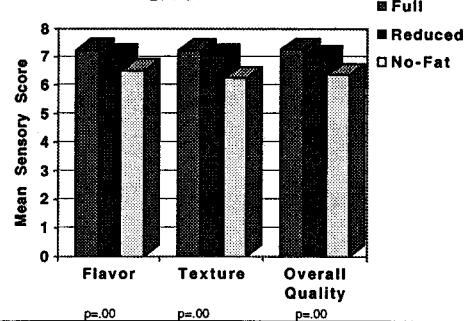
Cheese Sauce

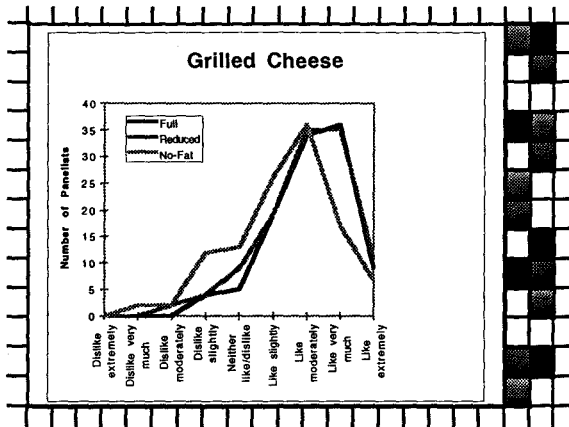


Cheese Sauce 1 Tablespoon

	Full-fat	Reduced-fat	No-fat
Calories	25	22	19
Fat	1.9 g	1.6 g	1.1 g
Protein	0.8 g	0.9 g	1 g
Calories from fat	68%	65%	53%

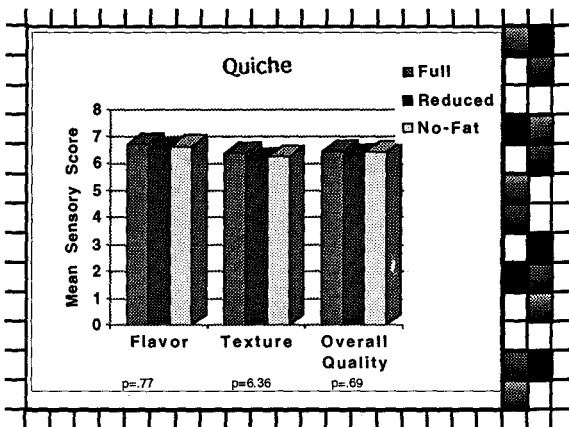
Grilled Cheese





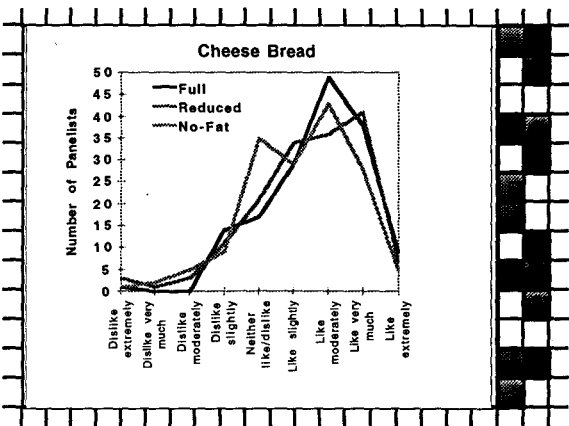
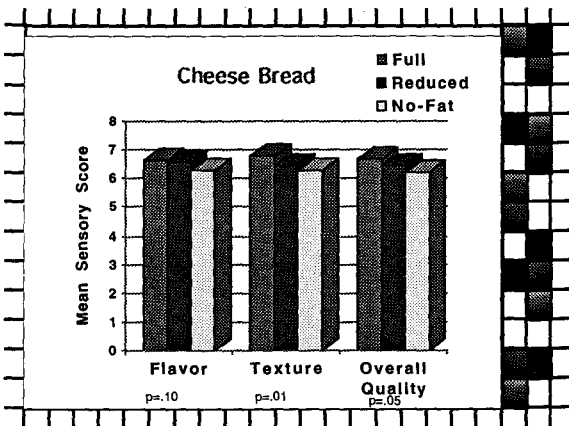
Grilled Cheese Sandwich

	Full-fat	Reduced-fat	No-fat
Calories	254	223	186
Fat	11.8 g	7.6 g	1.9 g
Protein	9.1 g	10.9 g	12.4 g
Calories from fat	41.7%	30.5%	9.2%



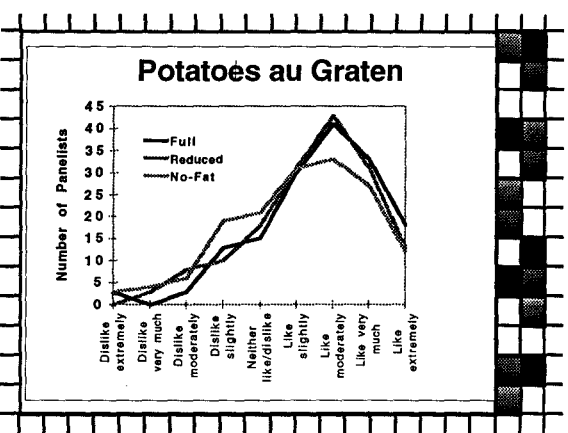
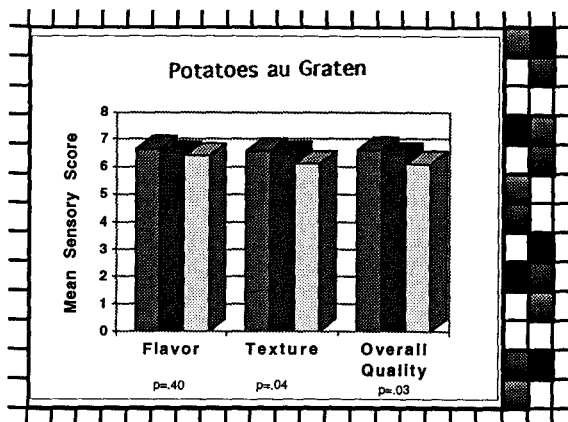
Quiche 1/6 pie

	Full-fat	Reduced-fat	No-fat
Calories	569	553	535
Fat	41.2 g	39.2 g	36.8 g
Protein	24.2 g	24.8 g	25.2 g
Calories from fat	65.2	63.7	61.9



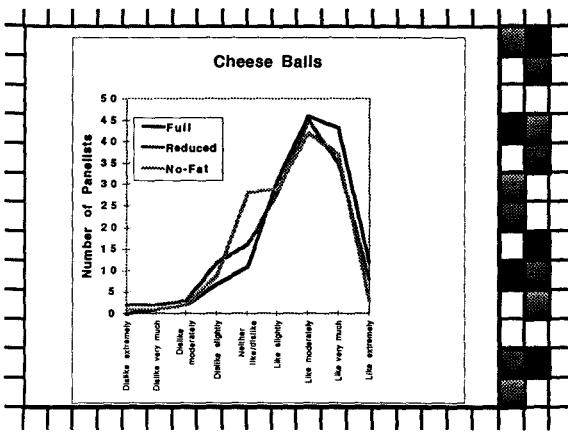
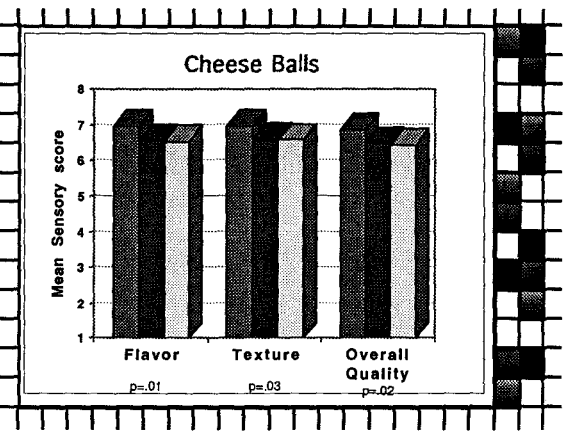
Cheese Bread 1 slice

	Full-fat	Reduced-fat	No-fat
Calories	135	130	123
Fat	3.2g	2.5 g	1.6 g
Protein	4.3 g	4.6 g	4.7 g
Calories from fat	21.3%	17.4%	11.5%



Potatoes au graten 1/2 cup

	Full-fat	Reduced-fat	No-fat
Calories	281	258	231
Fat	15.5 g	12.5 g	8.9 g
Protein	8.3 g	9.3 g	9.8 g
Calories from fat	50.0%	43.6%	34.6%



Cheese Ball

2 Tablespoon serving

	Full-fat	Reduced-fat	No-fat
Calories	68	63	54
Fat	6.7 g	5.7 g	4.5 g
Protein	1.7 g	2.0	2.2
Calories from fat	88.9%	81.9%	75.4%

Conclusions

- Reducing the fat when making cheese has an affect on color, flavor and texture.
 - No-fat cheese is a darker orange.
 - Rich mouthfeel decreased, some flavor compounds lost/decreased, saltier.
 - Firmer cheese, increase in rubbery texture.

- Some, but not all, recipes using low-fat cheese should be modified.

- May need to decrease salt.
- Cheese sauce types: Same ingredients but different preparation method.
- Surface melted cheese/grilled cheese: Minimize heat, use reduced instead of no-fat cheese.
- Grated & commingled with other flavorful ingredients: No change needed.
- Cheese ball: Increase liquid.

- Effectiveness in decreasing calories and fat by using reduced fat cheese can varies inversely with desirability.

- Quiche: no affect on quality & minimal changes in fat & calories.
- Grilled cheese: major affect on palatability, fat and calories.

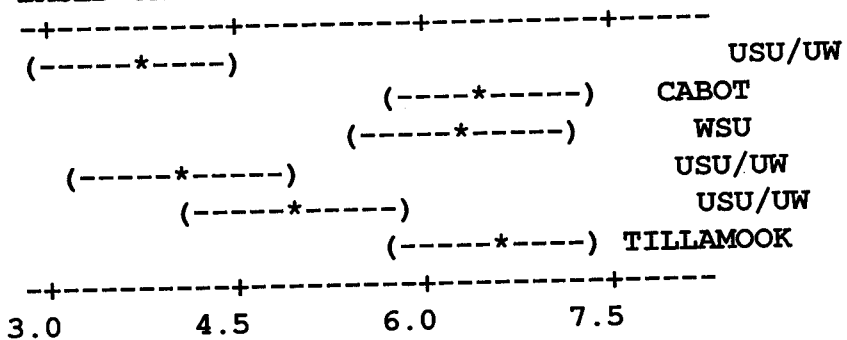
- To optimize decrease in fat and calories, recipes need modifications besides substitution reduced-fat cheese.

ANALYSIS OF VARIANCE ON BODY

SOURCE	DF	SS	MS	F	P
CODE	5	463.1	92.6	8.84	0.000
ERROR	330	3456.4	10.5		
TOTAL	335	3919.5			

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

LEVEL	N	MEAN	STDEV
96	56	3.714	1.626
471	56	6.509	1.500
745	56	6.286	1.474
774	56	4.027	1.574
930	56	4.929	7.163
973	56	6.554	1.413



ANALYSIS OF VARIANCE ON FLAVOR

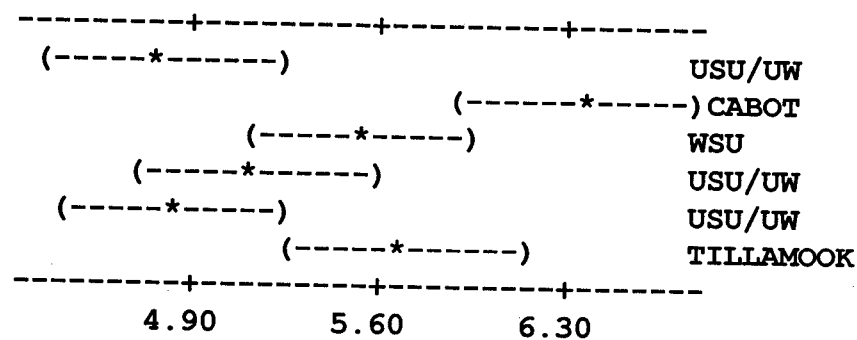
SOURCE	DF	SS	MS	F	P
CODE	5	98.60	19.72	6.92	0.000
ERROR	329	937.49	2.85		
TOTAL	334	1036.09			

alt

LEVEL	N	MEAN	STDEV
96	56	4.777	1.979
471	55	6.345	1.635
745	56	5.536	1.695
774	56	5.134	1.685
930	56	4.830	1.644
973	56	5.687	1.445

POOLED STDEV = 1.688

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV



ANALYSIS OF VARIANCE ON OVERALL

SOURCE	DF	SS	MS	F	P
CODE	5	270.04	54.01	19.75	0.000
ERROR	318	869.69	2.73		
TOTAL	323	1139.73			

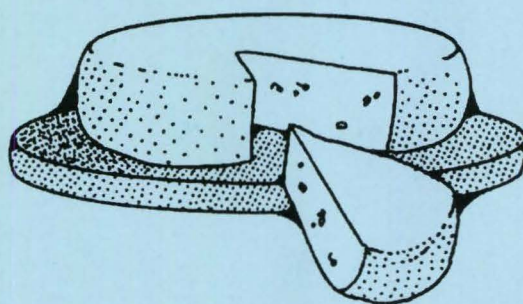
INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

LEVEL	N	MEAN	STDEV		
96	54	4.102	1.872	(---*---)	USU/UW
471	53	6.330	1.528		(---*---) CABOT
745	55	5.945	1.557		(---*---) WSU
774	54	4.574	1.733	(---*---)	USU/UW
930	54	4.269	1.739	(---*---)	USU/UW
973	54	6.046	1.455		(---*---) TILLAMOOK

POOLED STDEV = 1.654

4.0 5.0 6.0 7.0

**EFFECT OF AGING AND
BIOCHEMICAL INTERACTIONS ON
TEXTURE AND RHEOLOGY OF LOW
FAT CHEDDAR CHEESE**



**JOSEPH IRUDAYURAJ
UTAH STATE UNIVERSITY**

EFFECT OF AGING AND BIOCHEMICAL INTERACTIONS ON TEXTURE AND RHEOLOGY OF LOW FAT CHEDDAR CHEESE

Dr. Joseph Irudayuraj

PART I - Effect of Aging on Texture and Rheology

INTRODUCTION

Cheddar cheese is one of the most highly consumed cheese in United States, and has a substantial fat content, varying between 30% to 35% fat by weight. Problems such as poor texture, slow flavor development, and poor keeping quality are associated with reduced fat cheese. Although considerable work has been done in the past, information on the effect of aging on textural properties in low fat cheese is not available. Consumers acceptance of the food products are based on its sensory properties, texture, appearance, and flavor, which are evaluated by the information received from the five senses, taste, touch, sight, smell, and hearing. Texture measurements made by sensory panels are highly subjective, lacks reproducibility, and often are very time consuming. This led to the use of mechanical instruments for textural measurements, which are based on the rheological properties of food materials.

The objective of this research was to compare the textural attributes of different levels of reduced fat cheese. Also the casein degradation and resulting evolution of peptides will be studied using capillary electrophoresis to relate the peptide breakdown with the change in textural properties during aging. This study was focussed entirely on cheddar cheese. Three levels of reduced fat cheddar cheese, 25%, 50%, and 75% were studied and compared to full fat cheddar cheese.

Objectives

The specific objectives of this research were to:

1. study the effect of composition and age on the textural properties of three levels of reduced fat (25%, 50%, and 75%) Cheddar cheese as compared to full fat Cheddar cheese.
2. study the proteolytic activity of the four varieties of cheese to understand the difference in the rate of texture development during the aging process.
3. conduct sensory evaluation of the cheese to verify the correlation between the consumer response and instrumental textural measurements.

BACKGROUND

Texture or the body of cheese is an important parameter because, it is by this property that the consumer determines the identity and quality of a specific variety of cheese [Lawrence et al., 1987]. The texture of the different cheese varieties is different. This difference in the texture for different cheese varieties is related to the difference in proportion of the components of the cheese, rennet, milk, casein, moisture, lactic acid, sodium chloride, fat, and calcium [Lawrence et al., 1987; Prentice, 1992]. But as in most solid materials it is the structure of the cheese that influences the texture. The three major components contributing towards the structure of the cheese are casein (protein), moisture, and fat. The solid structure of cheese is primarily due to the cross linked casein-calcium phosphate network entrapped within which is fat and water [Lawrence et al., 1987; Jameson, 1990; Prentice, 1992; Anderson et al., 1993].

The structure of the cheese is altered with age, as a result of a series of chemical and microbiological changes, that affect the casein network. In an attempt to study the texture development of cheese through the aging process, proteolysis, casein breakdown, subsequent formation of peptides, have been extensively researched using advanced techniques of electrophoresis and HPLC. The texture development during the aging process can be characterized to proceed in two distinct phases [Lawrence et al., 1987]. Phase one, constitutes the first 7 - 14 days, when the rubbery texture of the young cheese is rapidly converted to smoother, more homogenized product as a result of break down of α_{s1} -caseins. Phase two involves a more gradual change in texture over the months as a result of continuing break down of α_{s1} -caseins and other caseins.

Chen et al. [1979], reported, from their study of textural properties of 11 different varieties of cheese, that fat did not contribute significantly to the variations in the textural attributes of cheese. Hence fat was considered to play a minor role in the classification of cheese [Fox, 1987]. However other studies [Emmons et al., 1980], showed that reduced fat cheese was harder and more elastic, described as rubbery, than the full fat cheese. This is considered a major impediment in the manufacture of low fat cheese and was discussed in the review of low fat cheese [Jameson, 1990]. The rubbery texture of low fat cheese is attributed to the increase in structural matrix per unit cross sectional area.

Effect of composition and age on texture of cheese

The three major constituents of cheese, which contribute towards its structure are casein, fat, and moisture. There has been considerable research published to show the effect of the composition on the textural properties of cheese. The solid nature of the cheese is contributed by the mesh like structure of casein, within which entrapped, are fat and moisture [Jameson, 1990; Prentice, 1992]. The percentage protein content reflects the firmness, which increases with the increase in protein content [Prentice, 1992]. At room temperatures fat in the cheese is present in both solid and liquid states and thus largely contributes to its plastic properties. The entrapped fat limits the deformation of the casein [Jameson, 1990], hence its absence results in greater elasticity. Also lower fat cheese has been reported to be harder [Jameson, 1990; Brandsma, 1994]. The majority of the moisture in cheese fills up the interstices between the fat and the casein matrix, while some water is bound to the casein and is immobilized. Consequentially the water acts as a low viscosity lubricant between the surface of the fat and the casein [Prentice, 1992]. Thus the increase in moisture content causes the cheese

Jack et al. [1993], studied the textural properties of 19 samples of cheddar cheese obtained from the market which varied in maturity and quality, to show the relevance of the rheological (TPA) and compositional data as possible predictors of sensory properties. From their study they reported that moisture content showed good correlation with perceived moistness and creaminess. Ash content was found to higher for drier and harder cheese. Also immature cheese showed higher recovery.

Proteolysis and its effect on texture cheese ripening

The texture development during the ripening process is said to follow two distinct phases [Lawrence et al., 1987], phase 1, constitutes the first 7 to 14 days, when the rubbery nature of the young cheese is rapidly converted into a smoother, more homogenized product, while phase 2 involves a more gradual change in the texture and is measured in months rather than days. As casein is the only continuous solid phase in cheese, the textural changes during the aging process could be attributed to its breakdown resulting from the proteolysis.

METHODS AND MATERIALS

Cheese Preparation

Full fat Cheddar cheese (FFCC), and the three levels of reduced fat cheddar cheese (RFCC),

25%RFCC, 50%RFCC, and 75%RFCC were made at the Utah State University Dairy lab. The cheese were made in triplicates. Due to the limitation on the number of vats available in the lab only two types of cheese could be made on any single day. The cheese were made in the following order. This schedule met the requirements for data analysis using ANOVA.

Replicate 1	week #1	25%RFCC & 75%RFCC
	week #2	50%RFCC & FFCC
Replicate 2	week #3	25%RFCC & FFCC
	week #4	50%RFCC & 75%RFCC
Replicate 3	week #5	75%RFCC & FFCC
	week #6	25%RFCC & 50%RFCC

The make procedures of the FFCC, and RFCC were slightly different. The primary difference being that during the preparation of the cheese, RFCC was milled at a higher pH of 5.95 as compared to 5.4 for the FFCC. This was done to decrease the protein breakdown in cheese during the aging process which results in a higher moisture cheese. A slow culture, to achieve a slow rate of acid development, necessary to maintain a strong buffering capacity was used for all cheese. Initially M-11 culture (Mesophilic Lactic Acid Producing Cocci), Waterford Foods Inc., was used for the cheese preparation, but its use had to be abandoned on detecting the presence of phage in the lab, which inhibited the curd formation cheese. Hence 11-M culture was replaced with CTD-blend (Mesophilic Lactic Acid Producing Cocci), Waterford Foods Inc., and the cheese made with 11-M repeated. The culture was grown in low fat milk (2%) for about five hours before being used.

About 280-290 lbs. Of Milk was used in each vat. Skim milk was standardized to 3.6%, 2.7%, 1.8% and 0.9% with cream using Pearsons equation to produce FFCC, 25%RFCC, 50%RFCC, and 75%RFCC respectively. 27 pounds of curd was hooped. The 25 lbs(approx) blocks of cheese were cut into 12 blocks after which they were vacuum packed and stored @ 4°C for aging.

Proximate Analysis

The percentage composition of fat, protein, moisture, NaCl and ash were determined using the standard methods outlined in the Standard Methods for the Examination of Dairy Products

[Marshall, 1992]. The components and the method of its determination are, fat - Babcock method; Moisture - forced draft oven method; protein - Kjeldahl method; NaCl - Coulometric titration method, ash - ash gravimetric method. All tests were done in triplicates.

Instrumental tests

Steven Farnells QTS-25 texture analyzer, was used for measuring the textural properties of cheese using the TPA technique. The instrument is operated with the help of an interactive windows based program, which also calculates and provides absolute values for product characteristics such as hardness, cohesiveness, etc.

A cylindrical probe of 1 inch diameter was used for all the tests. Cylindrical samples of 15 mm. diameter, and 20 mm. height [Jack et al., 1993], were chosen for all rheological tests. These samples are large enough to be representative of the whole and at the same time small enough to avoid the inclusion of structural irregularities, [Prentice, 1992]. All the samples were cut at 4°C using a cork borer, to prevent barreling of the cylinders. The samples were cut from the middle of the whole cheese blocks rather than the surface. The firmness of the cheese at the surface is relatively greater than that at the middle, due to the effect of surface drying, [Prentice, 1992].

All tests were done at room temperature, 21°C. The samples were allowed to equilibrate with the room temperature for 1 hour in a closed container, [Marshall, 1990]. TPA tests were done in replicates of five. The cheese samples were subjected to 60% compression with a cross head speed of 20 mm/min.

Statistical Analysis

ANOVA and regression analysis were used to find the statistical significance of the relationship between the percentage composition of the components and the textural properties of Cheddar cheese and the effect of age on the texture development.

RESULTS AND DISCUSSION

Textural attributes were measured for 1, 3, 7, 10, 30, 60 and 90 days old cheese. Hardness (Fig. 1), springiness (Fig. 2), chewiness (Fig. 3) were appreciably higher for 75% RFCC and decreased with increasing fat levels. Cohesiveness (Fig. 4) was also higher for reduced fat cheese but the relative difference between the different types of cheese was not much. During the aging process, while all

the textural properties decreased rapidly for the first 10 days springiness decreased slightly. Springiness decreased sharply between 30 and 90 days, with the springiness for 90 day old 75% RFCC being only about 47% of the 1 day old cheese. However, hardness, and cohesiveness increased for the 30, 60, and 90 days old cheese, while, chewiness, and springiness continued to decrease. Hardness and cohesiveness changed rapidly in the first 30 days but increased only slightly after that. The rate of change of textural properties was greatest for 75% RFCC and decreased with increasing fat content.

The rapid change in hardness during the first 10 days was due to the change in the rubbery texture to a more uniform and softer texture as a result of the breakdown of α_{s1} - caseins. During the later stages gradual change in hardness occurs due to the continuing breakdown of the α_{s1} and other caseins. The increase in hardness of low fat cheese can be attributed to the increase in structural matrix per unit cross sectional area. Reduced fat cheese

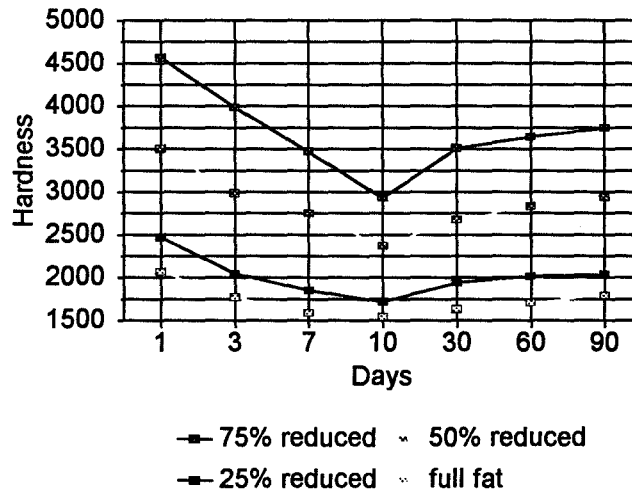


Fig 1. Effect of aging on hardness in cheddar cheese

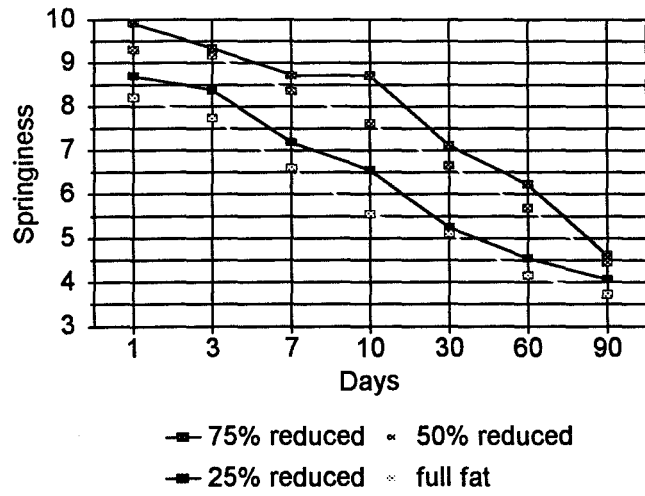


Fig 2. Effect of aging on springiness in cheddar cheese

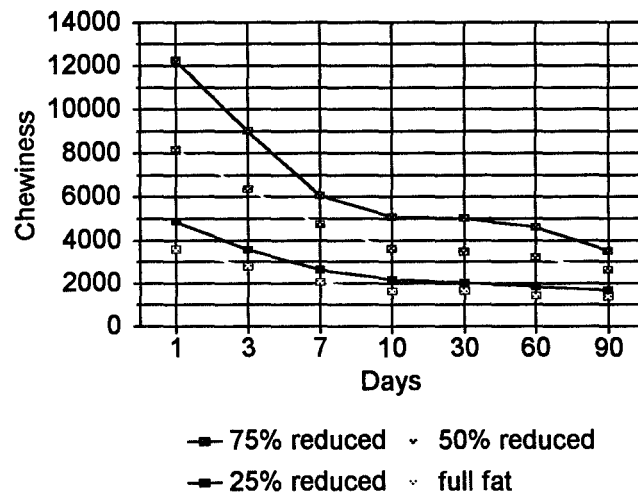


Fig 3. Effect of aging on chewiness in cheddar cheese

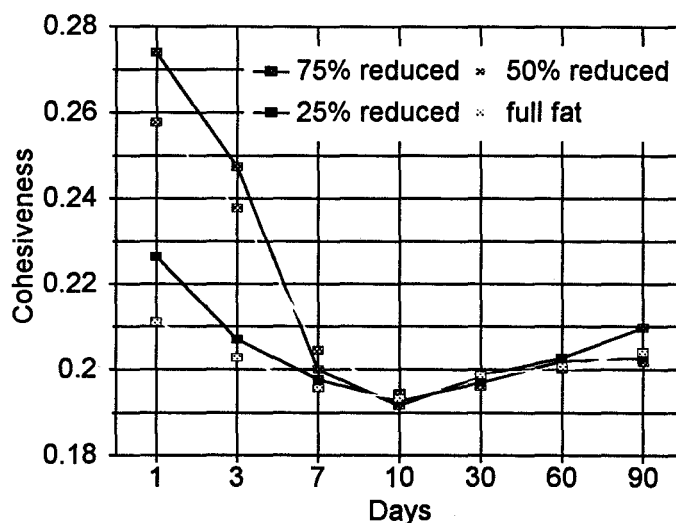


Fig 4. Effect of aging on hardness in cheddar cheese

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PART II : Aging Characteristics of Reduced Fat Cheddar Cheese using FTIR

ABSTRACT

Fourier transform infrared (FTIR) spectroscopy was used to study the characteristics of Reduced Fat Cheddar (RFC) and Full Fat Cheddar (FFC) cheeses during ripening. Strong and well separated bands at 1740, 1450, 1240, 1170 and 1115 cm^{-1} arising from fat, and at 1650 & 1540 cm^{-1} arising from protein were observed by using the frozen microtone samples. The spectra were affected by moisture and thickness of sample. Distinct changes in the bands of fat and protein in the spectra of RFC and FFC cheese samples were observed during ripening. There was also evidence that the intensity of bands at 1740, 1650 and 1540 cm^{-1} is proportional to the percent and types of fat and protein in cheese samples. This technique could be applied for a rapid composition analysis and characterization of the ripening process.

(Key words: Fourier transform infrared spectroscopy, reduced fat cheddar cheese, ripening)

INTRODUCTION

While the demand for reduced-fat cheeses continues to surge in the US (6), the production of these products still remains a challenge in many respects particularly with regard to flavour, texture and keeping quality. The significant change in cheese composition due to fat reduction modifies the microenvironment in the cheese which in turn modifies the bacterial growth patterns and enzyme activity (11). The final character of Cheddar cheese is determined not only by the initial composition of the product but also by the biochemical and chemical changes which occur during maturation (2). The effects of manufacturing process, composition of milk (such as fat level) and the biochemical events that occur during ripening play an important role in the production process. One of the important factors that will help in the process and product development is to understand the interaction of the different components in the cheese system. Of particular importance, is the role of moisture during the ageing of reduced fat cheese. The knowledge of the dynamics of water and its interaction with other compounds during the ripening process has not been fully understood.

Usually the methods to measure the components (fat, protein and moisture) of Cheddar cheese, such as Babcock for fat, Kjeldahl for protein and hot air oven method for moisture, are very cumbersome, slow and destructive to the sample. It is difficult to use these methods to measure and

monitor the biochemical changes (glycolysis, lipolysis and proteolysis) and the secondary catabolic changes (deamination, decarboxylation, beta-oxidation and even ester formation) that occur during production and ripening. Near-infrared (NIR) spectroscopy has been shown to be useful for direct, rapid and non-destructive quantification of major components in solid and semisolid foods, it was applied to analysis fat and moisture in Cheddar cheese (14). However this method always needs for large calibration sets and correlation methods.

In contrast to NIR, Fourier transform infrared spectroscopy (FTIR) has much to offer the analyst because specific bands may be assigned to specific chemical entities and statistical correlation methods are not always necessary, although they are not excluded and may be required in very complicated mixtures (4). FTIR technique was widely used to determine fat, moisture and protein in butter (19), meat (8), sweetened condensed milk (20), and high-fat products (21). It also can be used to monitor the oxidation of edible oils (22) and determine of low level *trans* unsaturation in fats (18). However, the application of FTIR to analyse cheese is limited. This may be due to the difficulty in sampling cheese and the effect of moisture in cheese on the resolution and reproducibility of spectra.

The main objective of this research was to investigate the potential application of FTIR spectrometer to analyse reduced fat Cheddar cheese during ripening. The specific objectives are 1) to develop a sampling method for analysis of cheese; 2) to study the absorption bands of fat, protein and moisture and their contents relevant to the respective spectral peak; and 3) to investigate the potential application of this FTIR technique in quality control of cheese production.

MATERIALS AND METHODS

FTIR Analysis

Cheese samples used for texture was used for FTIR studies. Sample for FTIR was prepared using the following procedure: Cheddar cheese samples from a block was cut into cylindrical shape with a diameter of 1.5 cm and frozen overnight at temperature of -80 °C. The frozen sample was microtoned to a thickness of 16 µM with an IEC Minotome (IM236, International Equipment Co.) and stored at -80 °C for use in FTIR. The frozen Cheddar cheese sample was then placed on the surface of silver chloride crystal supported by a sample holder which was kept in the path of sample beam in the spectrometer.

ATR measurements were obtained with a Spectra-Tech continuously variable angle ATR

attachment with a KRS5 crystal (45°, 50 × 20 × 3 mm parallelogram). The spectra were studied with the incident angle set at 45°. Wetted cheese samples were sliced about the same size of sample crystal size with the thickness of 1-2 mm. Then the sample was spread uniformly over one face of the crystal.

Spectroscopic data were collected using a Mattson Polaris Icon Fourier Transform Infrared spectrometer equipped with a TGS detector. The region from 4000 cm⁻¹ to 400 cm⁻¹ was scanned with a resolution of 1, 4 and 8 cm⁻¹ (16 or 32 scans/sample). The mirror velocity was 4 cm⁻¹/s, and interferograms were co-added before Fourier transformation and triangular apodisation was employed.

RESULTS AND DISCUSSION

Spectra of Cheddar Cheese

Typical spectra of full-fat and reduced-fat (75%) Cheddar cheese (about 10 wk old) is shown in Fig. 1. Both of these spectrum have a good resolution and signal to noise ratios. In the spectra, a number of bands arising from fat can be seen, with the strongest being at a wavelength of 1744 cm⁻¹ arising from C=O (ester) and at 2930 and 2850 cm⁻¹ arising from C-H stretch vas and vs of -CH₂- respectively, as C-H stretching vibrations, associated with methyl and methylene groups, were generally observed in the region between 2960-2850 cm⁻¹ (17). Similar to the work reported by Belton et al (5), fat related bands occurring at 1477-1400 cm⁻¹ (C-H bend), 1240 cm⁻¹ and 1170 -1115 cm⁻¹ (C-O stretching) were also obtained. Both of the spectra showed a well separated strong signal from protein at about 1535-1545 cm⁻¹ arising from amide II (7) and 1650-1658 cm⁻¹ from amide I (9). Belton et al. (4) also reported that well separated bands at 1650 and 1540 cm⁻¹ arising from protein in confectionery products were obtained using FTIR technique with the aid of photoacoustic sampling cell.

But in their work, the presence of a water band at 1650 cm⁻¹ obscured the amide I band if the wetted material is used for the ATR measurements. Although usable spectra from wetted systems were obtained only with ATR, a useful calibration graph could not be constructed because the amide I band was swamped by the large water peak and in some instances the amide II was superimposed on several sloping baseline and was considerably offset (4). The water obscuration greatly affected the spectra using ATR and no significant signals could be observed for fat and protein because of the very low signal-to-noise ratio in our work.

However the effects of water obscuration at 1650 cm^{-1} seemed to only slightly affect the spectra from microtone frozen cheese samples by comparing the masking or modification of the strong broad bands of moisture at $3000\text{-}3600\text{ cm}^{-1}$ (Figure 2). The moisture bands also affected the multiple NH bands in the $3330\text{-}3060\text{ cm}^{-1}$ region since the amide group can bond to produce dimers, with a *cis* conformation, and polymers, with a *trans* conformation (17). But this can be eliminated by collecting the spectra after a period of time when it is stable.

The Effects of Time and Resolution

Liquid water has a very strong, broad band which is totally absorbing in the $3700\text{ to }3100\text{ cm}^{-1}$ range, and a weaker band around 2000 cm^{-1} and another strong band at 1640 cm^{-1} (15). At about 800 cm^{-1} , water stops transmitting altogether. Comparison of the spectra of the cheese sample obtained immediately and after some time (Fig. 2), indicated that strong broad bands at $3100\text{-}3700\text{ cm}^{-1}$ and $600\text{ to }800\text{ cm}^{-1}$ during the first 5-8 mins arising from moisture could result in masking or modification of absorptivity of other components, especially the bands in 3180 and 3300 cm^{-1} region arising from multiple bands of N-H stretching vibrations associated with hydrogen bonding (17). It also caused a small increase at band of 1650 cm^{-1} . However after 10 mins, a more stable spectra with a better resolution was obtained. This may be due to the evaporation of free or surface moisture in the cheese sample during measurement. Figure 3 shows the plot of moisture loss by natural evaporation. The results indicate that about half of the total moisture in Cheddar cheese sample is lost after drying the cheese sample for 5 days. Hence, to measure fat and protein, the spectra should be collected after 10 mins of placement of the sample in the holder in order to minimize the distortion due to moisture effects.

The resolutions of 1 , 4 and 8 cm^{-1} were used to collect the spectra of ten week aged 50% RFC cheese. The results in Figure 4 show that smaller the resolution, the more sensitive are the bands. Hence at a resolution of 1 cm^{-1} , the spectrum was very sensitive and at 8 cm^{-1} it was very rough; therefore a resolution of 4 cm^{-1} was used in this work.

Effects of Sample Thickness

The sample thickness has a direct effect on the spectra of Cheddar cheese. The spectra of 50% reduced-fat Cheddar cheese sample at a thickness of $16\text{ }\mu\text{m}$ and $32\text{ }\mu\text{m}$ is given in Fig. 5. The

infrared intensities at different bands is related to the thickness of the sample according to the following equation (1):

$$A_i = \int_{\nu} k(\nu) d\nu$$

Where,

$k(\nu) = (1/CL) \ln(I_0/I)$ is the integration covering the finite band width

C: molar concentration

L: path length of the sample

I_0 : intensity of the incident light

I: intensity of the light leaving the sample

The thickness of Cheddar cheese sample affected the path length of the sample. Therefore, in order to get reproducible results, the samples must be examined at the same thickness in the quantitative analysis of cheese components. The thickness of 16 μm was used in this work.

Spectra of FFC and RFC Cheese

Figure 6 compares the Spectra of 12 week aged RFC (25%, 50% and 75% reduced) with that of FFC cheese. The spectra were collected after 10 mins from a 16 μm thick sample. Strong and well separated bands arising from fat (1744 cm^{-1} , 2930 and 2850 cm^{-1}), protein (1650 and 1540 cm^{-1}) could be observed for all cheese samples. The spectra of different fat level Cheddar cheese samples have the same absorption bands, but the intensity was different. Comparison of the results of proximate analysis and the intensity of bands at 1744 and 1540 cm^{-1} mostly arising from fat and protein respectively (Fig. 7), one could hypothesize that the intensity of bands at 1744 and 1540 cm^{-1} was proportional to the contents of fat and protein in Cheddar cheese. This indicated that all components could be quantified simultaneously by giving suitable standards or spectral ranges.

A noticeable difference in the spectra of bands in the range 1100-1300 cm^{-1} arising from C-O stretch vibrations of fat for FFC and RFC cheese samples was observed. The intensity ratio of bands in the range between 1170 cm^{-1} and 1240 cm^{-1} was found to be proportional to the level of fat level in cheese. It is the highest for FFC cheese and lowest for 75% RFC cheese.

Spectra of Cheddar Cheese During Ripening

The spectra of FFC (Fig. 8a) and 25% (Fig. 8b), 50% (Fig. 8c) and 75% (Fig. 8d) RFC cheese

samples were collected during different periods of ripening.

The spectra of FFC and 25% RFC cheese have a comparable peak shape and intensity at different bands during ripening; however, those of 50% and 75% RFC cheese were significantly different from that of FFC cheese. These results agree with Olson and Johnson (13) who reported that when the fat was reduced by 25% in Cheddar cheese, it compared well with its full-fat cheese; but reduction of 50% or greater resulted in cheese of lower flavour and physical properties. It should also be noted that when the Cheddar cheese is young (about 7 wk), the intensity of band 1650 cm^{-1} was much lower than that of old cheese (12-15 wk), especially for FFC cheeses. A change in the position of peak around $1630\text{-}1660\text{ cm}^{-1}$ may be caused by the change of amide I group because of the degradation of various caseins (α_s and β) during cheese ripening (3).

Studying the changes in protein composition during long-term storage of traditional Cheddar and stirred curd Cheddar cheese, Basch et al. (3) noted that as the age of the cheese increased, there was a partial breakdown of caseins into fragmentary products and peptides: α_{s1} -casein degraded first while β -casein persists for a long time and α_{s2} -casein and para- κ -casein were intact beyond 70 weeks before degrading. Using a spectral database, Sarver and Krueger (16) proved that peak positions of 1660 , 1653 , 1650 and 1634 cm^{-1} were due to β -turn, α -helix, other, and β -sheet conformation, respectively. Therefore the degradation of caseins in Cheddar cheese changes the amount of amide I group and the conformation of secondary structure of caseins. The band at 1650 cm^{-1} had a higher intensity for young RFC cheese than FFC cheese at a similar ripening stage. This was in agreement with the work of Nauth and Ruffie (12) who deduced that in reduced fat cheeses, proteolysis of α_s -casein is always greater and faster (3) than any other protein. The intensity and bandshape at 2850 and 2930 cm^{-1} arising from C-H stretch was and vs of $-\text{CH}_2-$, and 2960 cm^{-1} from C-H stretch was of CH_3 also change during ageing because of the release of fatty acids by lipolysis (12).

The strong band at 3280 cm^{-1} arising from broad, intermolecular hydrogen bonded, O-H stretch (17) due to $\text{O-H}\cdots\text{X}(\text{X}=\text{N}, \text{F}, \text{O} \text{ etc})$ structure was observed in the spectra of all cheese samples and changed during ripening. Intermolecular hydrogen bonding involves association of two or more molecules of the same or different compounds. The hydrogen bonding existing in bound water, amino acids, polypeptides, ketone and alcohol type substances was attributed to the change of intensity of this band. Hence the intensity at band of 3280 cm^{-1} changed differently for RFC and FFC cheese.

CONCLUSION

FTIR spectroscopy technique was applied to study the ripening of Cheddar cheese of different fat levels. By controlling the thickness of the sample and analysis time, a reproducible spectra can be collected. The strong bands at 3300, 1745, 1650, and 1560 cm^{-1} arising due to moisture, fat and protein was observed in the spectra of Cheddar cheese sample. From the spectra of Cheddar cheese sample, information regarding the contents and breakdown of fat and protein in cheese during ripening could be obtained. FTIR technique has the potential to be used for a rapid analysis of fat, protein and moisture in cheese. Dynamic monitoring of the status of cheese during manufacturing and ripening may be possible using FTIR technique. This will help us to understand the chemical reaction occurring in cheese and produce an acceptable product.

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22. Van de Voort, F.R., A. A. Ismail, J. Sedman and G. Emo. 1994. Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy. *JAOCS.* 171(3):243.

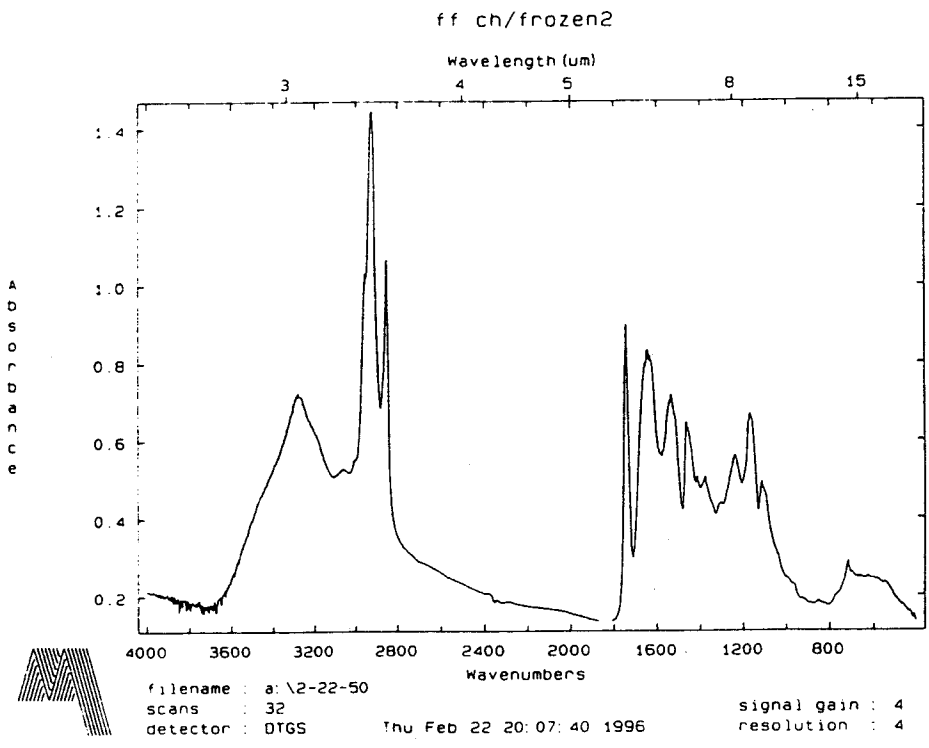
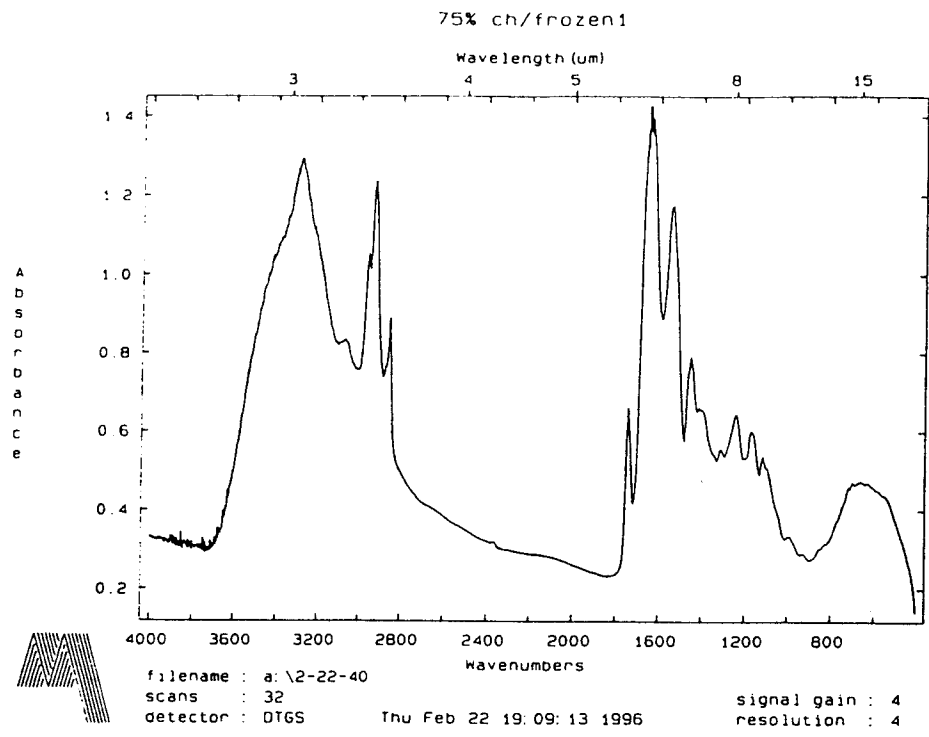


Figure 1. Typical spectra of Cheddar cheese (about 10 wk aging time). a. 75% reduced fat; and b. Full fat.

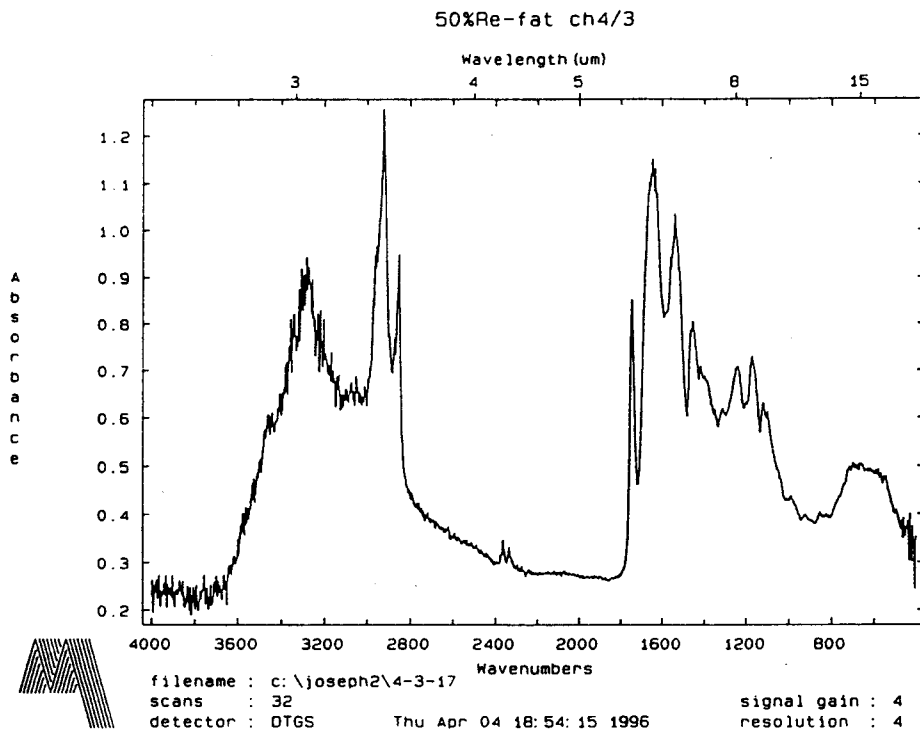
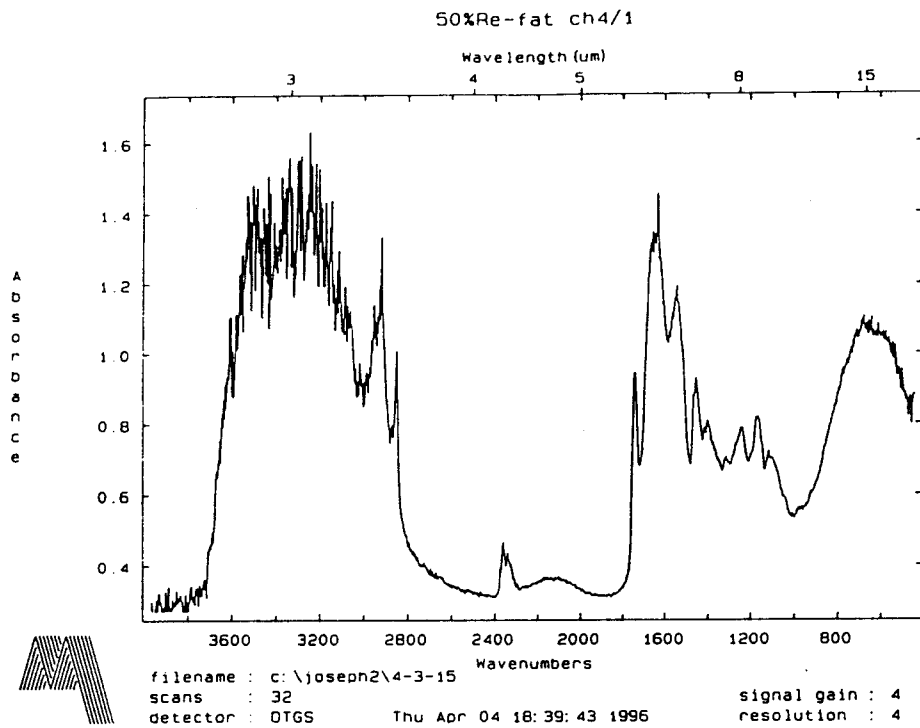


Figure 2. The spectra of 50% reduced fat Cheddar cheese (about 18 wk) collected immediately, a; and after set for 15 mins in air, b.

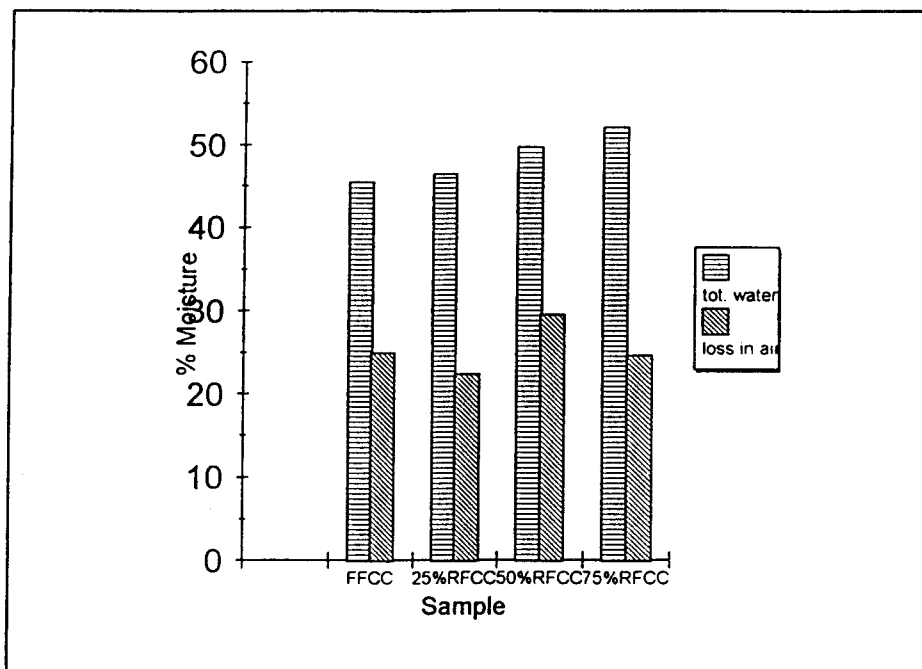


Figure 3. The loss of moisture in cheese sample by drying in the air.

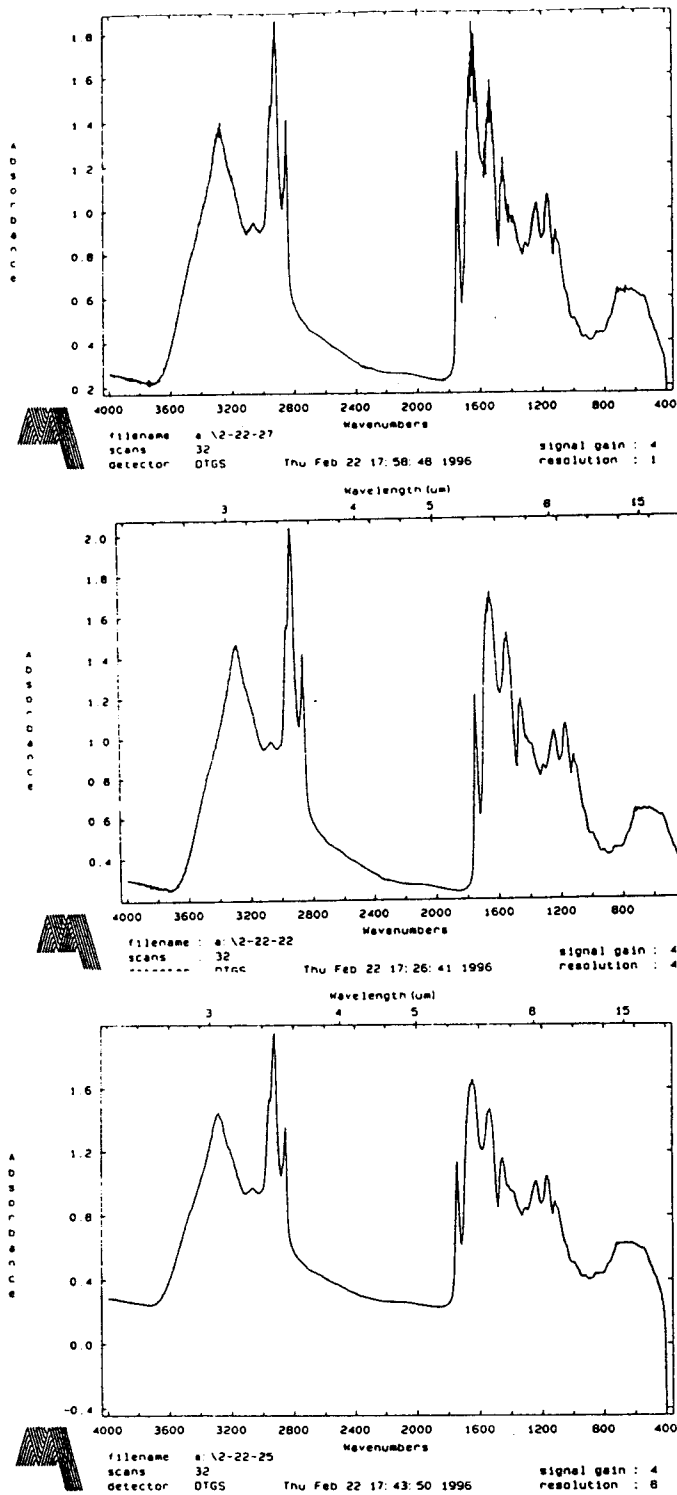


Figure 4. The spectra of 50 % RFC cheese collected with resolution of 1 cm^{-1} , a; 4 cm^{-1} , b; and 8 cm^{-1} , c.

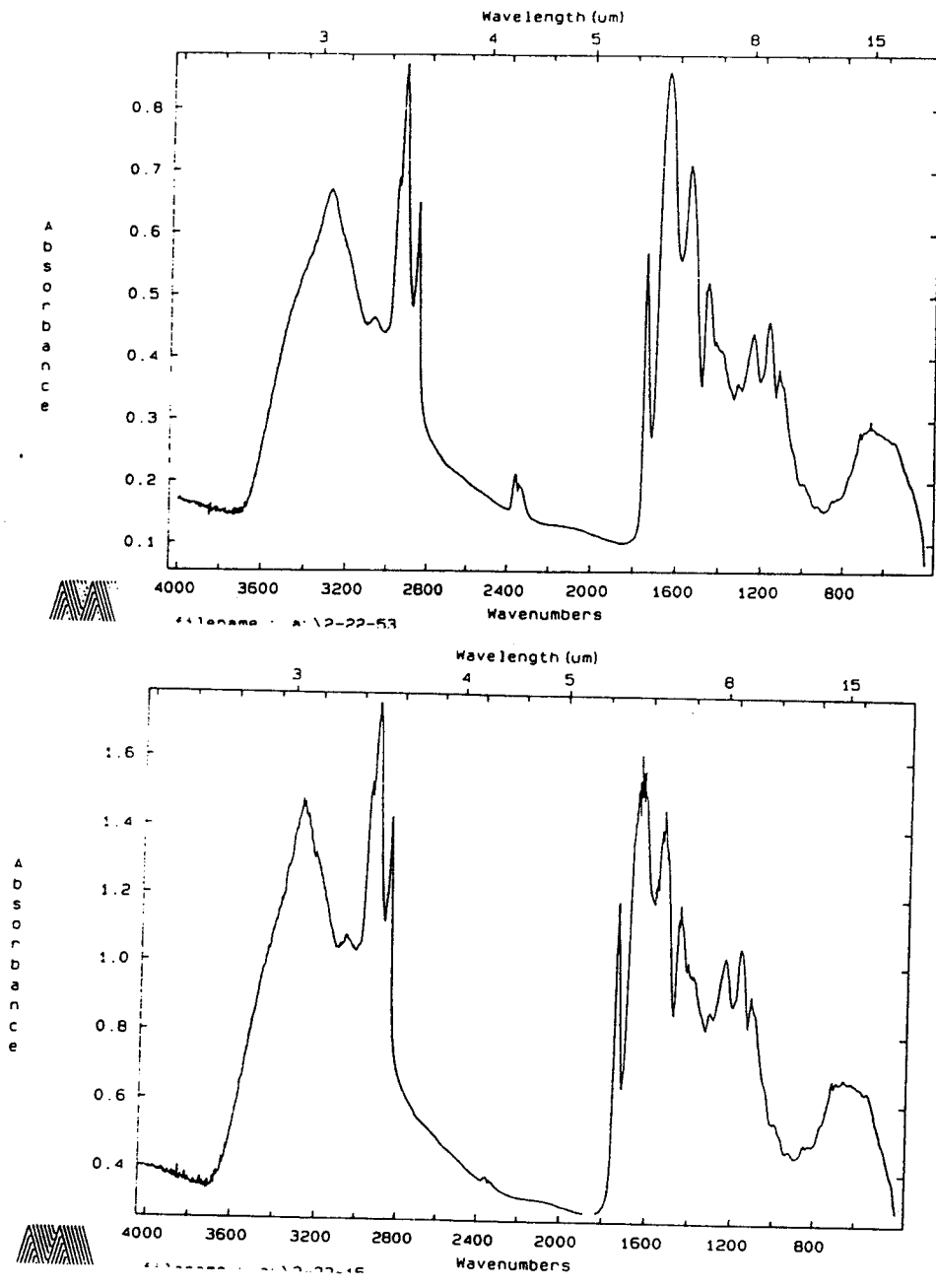
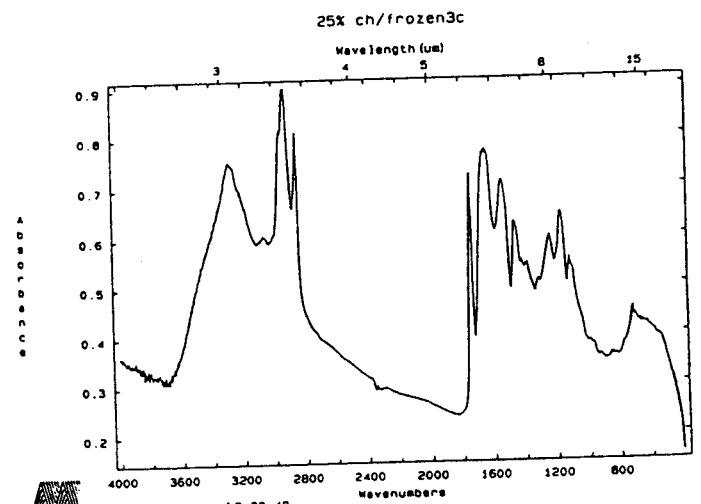
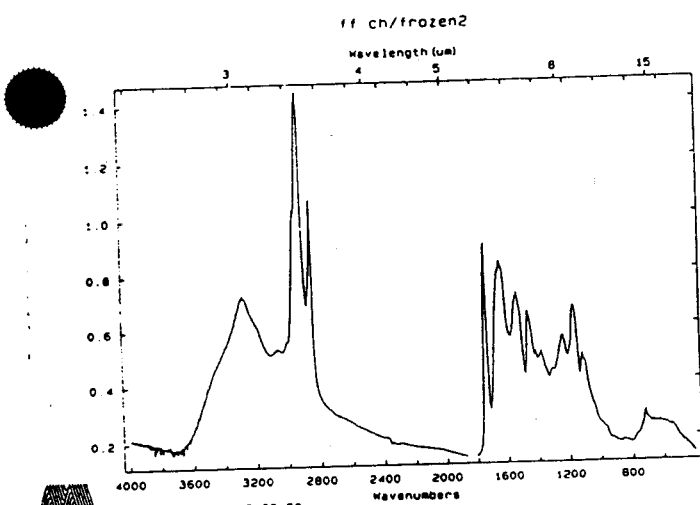
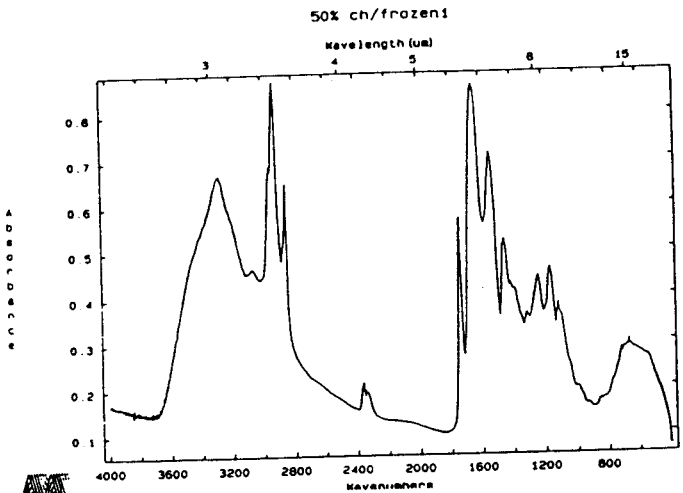
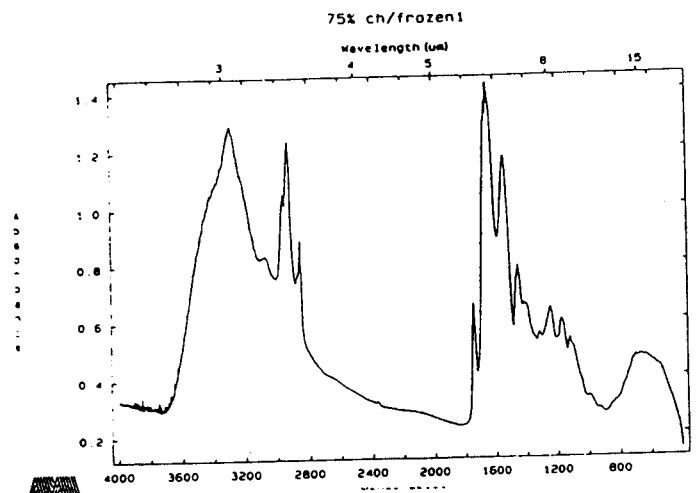


Figure 5. The spectra of 50% RFC cheese (about 12 wk) measured with a sample thickness of 16 μm , a; and 32 μm , b.



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 signal gain : 4
 resolution : 4
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filename : a:\2-22-48
 scans : 32
 detector : DTGS
 signal gain : 4
 resolution : 4
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Figure 6. The spectra of 12 wk aged Cheddar cheese from milk with 75% reduced fat, a; 50% reduced fat, b; 25% reduced fat, c and full fat, d.

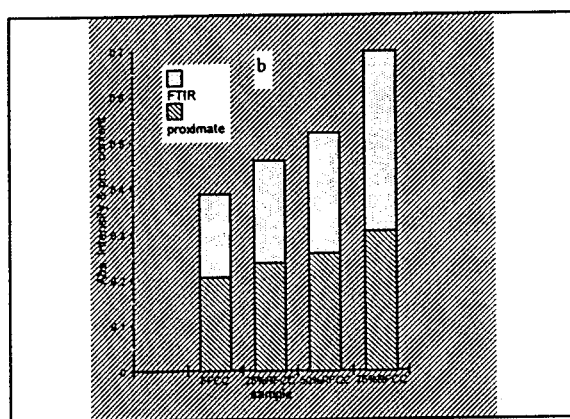
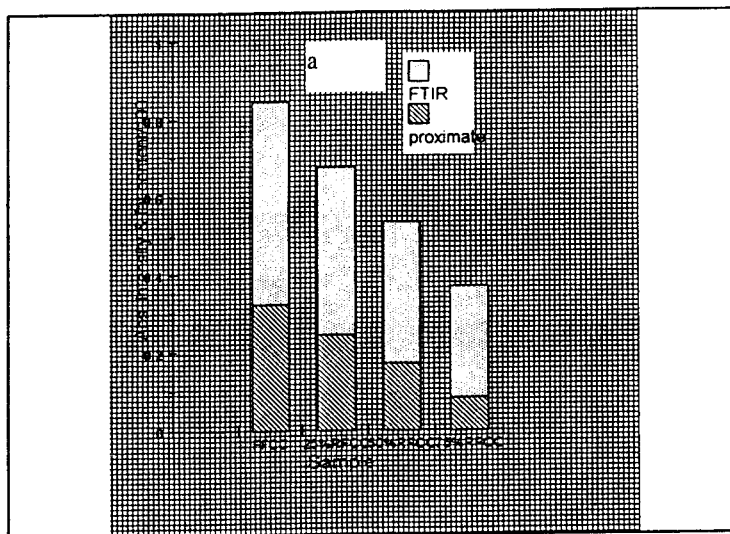


Fig. 7. Comparison of the results of proximate analysis and the intensity of bands at 1744 (a) and 1540 (b) cm^{-1} relevant to fat and protein respectively.

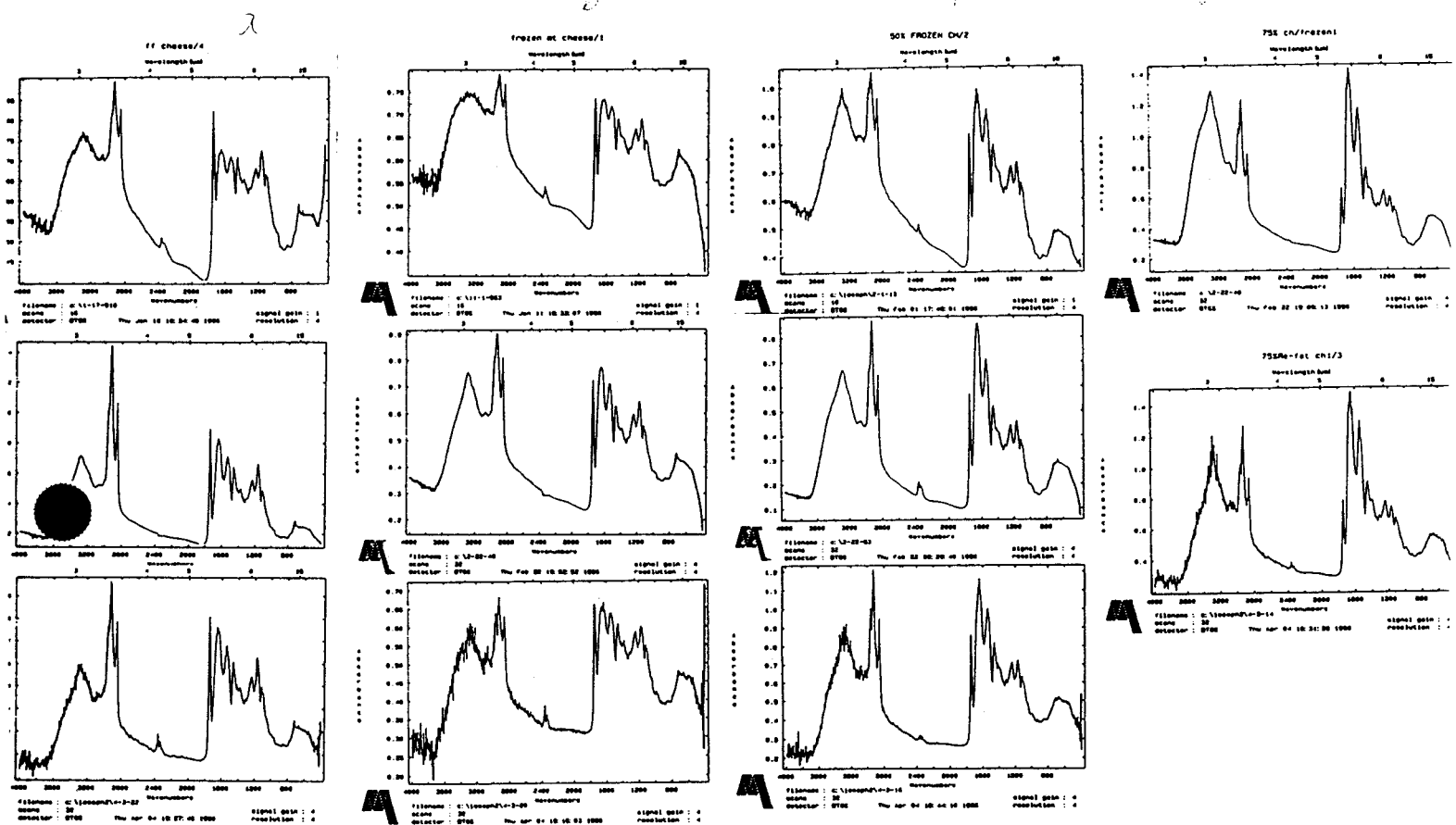
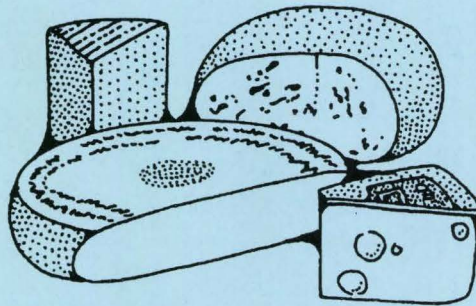


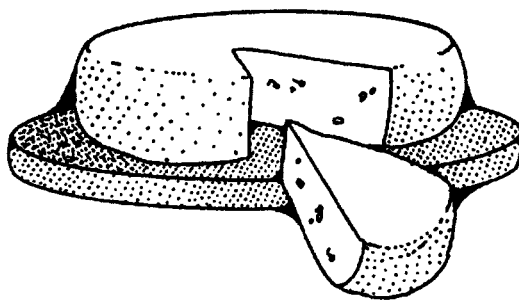
Figure 8. The spectra of Cheddar cheese at age range of 7 to 18 wk, which collected from FFCC, a; 25% RFCC, b; 50% RFCC, c; and 75% RFCC, d.

MEASURING STRETCH OF MOZZARELLA CHEESE



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UTAH STATE UNIVERSITY

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INTRODUCTION

One of the most important characteristics of Mozzarella cheese used as pizza topping is the ability to stretch when melted. Although the term "stretch" lacks a precise rheological definition, it refers to the capacity of the melted cheese to form fibrous strands which extend under tension (6). Since cheese manufacturers have a difficult time relating to rheological properties and textural scientists have little use for qualitative evaluations, many approaches have been used to evaluate stretch. They range from the subjective fork test commonly used by cheese manufacturers, to the purely objective empirical tests found in the laboratory (Figure 1).

We will examine some of these tests and review work on an additional stretch test being developed at Utah State University.

Fork Test

The fork test is the most common method used in the cheese industry to evaluate the ability of Mozzarella cheese to stretch. It is a qualitative test that is performed by placing ground cheese on a thawed pizza crust containing pizza sauce. The pizza is then baked in an oven until done and allowed to sit for a short time before a fork is inserted into the melted cheese. The fork is then raised vertically until the cheese strands break. The "stretch" is defined as the strand length at the time the strands break.

The type and size of crust, amount of sauce, oven style, and baking parameters are determined by the customer. Crust sizes range from 8 to 14 in, sauce amounts from 2 to 5 oz, cheese amounts from 8 to 12 oz, bake times from 4 to 6 min, and temperatures from 400 to 550°F or higher. A typical test might use a 12 in pizza crust, 4 oz of sauce and 10

oz of ground cheese. The pizza would be baked from 4 to 6 min at 500°F and allowed to sit from 0.5 to 1 min before inserting the fork and stretching the melted cheese.

Since test parameters are dictated by the customer, test conditions have never been standardized. However, many manufactures use a 10 to 20 point scale in addition to a length measurement in an attempt to better evaluate their cheese. Scale units and their significance are proprietary and differ among companies.

Although the type of crust, amount of sauce and cheese, and the bake conditions are controlled, most technicians perform the fork test differently. Where and how the fork is inserted, tine orientation, the amount of tine covered by the cheese and the speed used to lift the cheese varies. In addition, defining a strand or the point at which the “majority” of the strands break depends on the experience and judgment of the technician. For these reasons, it is not uncommon for evaluations of the same cheese to differ.

Even with this built-in subjectivity, the fork test has served the Mozzarella cheese industry well. The only information the customer wants to know is that the cheese they purchase melts and stretches the way they want it to. The manufacturer requires the same information so that they sell the cheese the customer wants. Since the fork test provides this qualitative information, it is the most common test used to evaluate stretch in an industrial setting.

Helical Viscometry

Helical viscometry has been successfully used to evaluate the stretch characteristics of melted cheese (5,7,8,12). The test measures the torque on a rotating t-bar spindle as the spindle is raised through the cheese. As the t-bar rotates, fibrous cheese strands

accumulate on the spindle and increase the torque on the shaft. The maximum resistance recorded during the spindle's pass through the cheese is recorded and is expressed in relative units of full-scale response to the viscometer spring.

Helical viscometry, along with the melt test of Olson et al. (10), has been used to demonstrate the inverse relationship (Figure 2) between stretch and melt as Mozzarella cheese ages and the rapid loss of stretch characteristic that takes place during the first 7 to 14 d (8). Viscometer profiles have been used to compare the stretch characteristics of different types of cheese (9)(Figure 3), and are currently being used to compare the age related changes between part skim and low fat Mozzarella cheeses (Figure 4). From these latter profiles it appears that proportionally more stretch characteristic is lost in low fat Mozzarella cheese than part skim Mozzarella cheese during the first 14 d refrigerated storage (5).

Uniaxial Horizontal Extension

AK, et al. (3) developed an empirical method that measures the tensile properties (stretching characteristics) of Mozzarella cheese pulled horizontally (Figure 5). The horizontal orientation simulates conditions comparable to baking.

Thin dumbbell-shaped pieces of cheese are preconditioned in an incubator to the desired test temperature. The cheese is then placed horizontally in a heated oil bath and secured in place by clamps which are attached to the large ends of the cheese. One clamp remains stationary while the other is attached to a load cell by a cable and pulley system. When the apparatus is activated, the cheese is pulled by the ends causing it to stretch in

the middle. Test temperatures higher than 40°C cause the samples to sag, so most tests are performed between 10°C and 40°C.

Fracture strain (Hencky strain corresponding to the stress at fracture), fracture stress (tensile strength), and deformability modulus can be generated at different temperatures, ages of cheese and deformation rates. Typical graphs (Figure 6) show that fracture strain increases during the first 14 d then remains fairly constant throughout 28 d refrigerated storage. Fracture stress increases slightly then decreases after 14 d, and the deformability modulus decreased throughout 28 d storage. Coupling this information with proteolytic data provides an additional insight into stretch characteristics.

Vertical Extension (Pizza Base Template) / Compression Elongation

Apostolopoulos (4) has developed a tensile test that better represents the way a consumer assesses the stretchability of cheese used as pizza topping (Figure 7; left, a & b).

A circular plate is used as a template to hold the pizza crust. A smaller circular piece (with a vertical rod attached) is cut out allowing the center of the template to be raised independently of the edge. A similarly cut pizza crust is placed on the template and a standard weight of cheese sprinkled on top of the crust. The complete apparatus is heated in a microwave oven for 15 s to melt the cheese. On removal from the oven, the vertical rod is attached to the crosshead of a tensile testing machine and pulled vertically, stretching the melted cheese.

The extensibility of the cheese is taken as the distance of travel until all the cheese strands break. A comparison to sensory evaluations indicated that the extensibility test correlated well to the way consumers evaluate cheese on a pizza.

A more fundamental test was also developed to evaluate the elongation properties of melted cheese (Figure 7; right, a & b). A non-lubricated cylindrical cheese sample was placed on a hot plate and covered with a metal cap until the sample temperature reached 65 °C. After the cheese melted, it was immediately compressed by another plate attached to a tensile testing machine at 20 mm/min. The elongation viscosity was then calculated and used as an index of the ability of the cheese to form strings when it is stretched.

Uniaxial Vertical Extension

AK and Gunasekaran (1) modified the uniaxial horizontal extension so that samples were stretched vertically and at higher temperatures (Figure 8). One end of the dumbbell shaped sample is attached to a load cell by a clamp while the other end is attached to a weight. The untempered sample is then lowered into a temperature-controlled oil bath and sample elongation measured by an infrared emitter and detector system.

Unlike the tests described above, the strain and stress rates are not constant since the temperature of the sample increases throughout the test; therefore, results are reported in the form of transient elongation (TE) viscosity (Figure 9).

Vertical uniaxial extension showed that the TE viscosity of Mozzarella cheese decreased as the strain rate and sample temperature simultaneously increased during a test. It was noted that proteolysis during the first month of storage did not effect the TE and was perhaps an indication that the instrument was not sensitive enough to detect slight structural changes under the test conditions.

Squeezing Flow Method

AK and Gunasekaran (2) have recently adapted a Instron Universal Testing Machine for lubricated squeeze flow tests (Figure 10). In concept, it is similar to the fundamental compression test of Apostolopoulos (4) and provides much of the same information. Cylindrical cheese plugs, cut parallel to the cheese fiber orientation, are tempered in an oven then placed between an upper and lower compression disk. The upper disk is then driven downward at a constant speed squeezing the cheese between the plates. A load cell records the amount of force required to deform the cheese in this manner.

Deformability modulus, compressive relaxation modulus, relaxation times, elongation viscosity, and biaxial stress growth coefficients can be calculated from the data.

Probes

It should be mentioned that the shape of the probe (assuming a tensile-type test) determines the type of data obtained and its relevance. For example, the fork test takes its name from the type of probe used, i.e. a fork. Since forks come in all shapes and sizes, it stands to reason that the information obtained by using a wide plastic fork with short tines might differ from that obtained when using a thin, long-tined stainless steel fork.

Recently, Pena et al. (11) evaluated three probe styles (Figure 11) for vertical tensile testing and correlated the data with sensory evaluations. They determined that an open-wire configuration best correlated with sensory panel results.

USU Tensile Test

A tensile test for measuring the stretch of Mozzarella cheese is being developed at Utah State University. The test not only provides classical rheological data but also parameters useful to the cheese manufacturer.

Ground cheese is placed into a stainless steel cup and tempered in a water bath until melted (60 to 90°C). The cup is then removed and placed in a water-jacketed holder mounted to a Stevens Farnell Quality Testing System. After the probe is lowered into the sample, the cup is rotated into position and the cheese pulled vertically until all the strands break or the beam stroke maximum is reached.

The stretch profiles of three cheeses; low fat cheddar cheese, 13 months; part skim Mozzarella cheese, 26 d; and low fat Mozzarella cheese, 38 d, appear in Figure 12. Load increases as the probe is pulled vertically through the melted cheese and exits the surface. It then declines at various rates and either reaches a plateau as cheese strands elongate or returns to base-line if they break.

Experience with helical viscometry suggests that apparent viscosity measures the viscosity of the melted cheese so long as the t-bar remains beneath the surface while stretch characteristics familiar to the consumer occur after the t-bar leaves the surface. Therefore, in addition to the classical rheological parameters that are generated from the tensile test, we also look at the profile tail sections for information.

As cheese fibers form around the probe, the load on the cell increases until a maximum value is reached and the probe exits the cheese surface. This value, melt strength, is analogous to the peak load and is an indication of strand forming ability.

(Figure 13). Stretch length (Figure 14) has been used as the standard measure of stretchability and is represented here as the distance from the maximum melt strength until strand failure, or the maximum beak stroke is reached. In this example, low fat cheddar and part skim Mozzarella cheeses stretched the entire stroke distance (31 cm), while low fat Mozzarella cheese did not stretch well (5 cm). How well cheese fibers stretch is determined by the ability of the cheese strand(s) to remain together as a cohesive mass while being pulled. The term, stretch quality, is used to describe this characteristic and is calculated as the mean value (g) of the load exerted as the strand elongates from 7 to 15 s. During this time, surface-cheese influence is minimal as indicated by the flatness of the curve.

Table 1 shows typical data from tail section analysis. Part skim Mozzarella cheese and low fat cheddar cheese had melt strengths of 191 and 143 g respectively, while low fat Mozzarella cheese had a melt strength of 127 g. Both the low fat cheddar and part skim Mozzarella cheeses stretched the entire length of the stroke (31 cm) while the low fat Mozzarella cheese had almost no stretch (5.2 cm). Stretch quality followed melt strength, although this is not always true. Part skim Mozzarella cheese had a stretch quality of 21.3 g while the low fat cheddar cheese had a value of 10.0 g. Low fat Mozzarella strands had almost no stretch quality (0.9 g).

By utilizing information in the tail sections of tensile profiles and using terms such as melt strength, stretch length, and stretch quality, tensile testing equipment can provide useful qualitative as well as empirical information for both cheese manufacturer and textural scientists.

ACKNOWLEDGMENTS

This research was supported by the National Dairy Protein Promotion and Research Board and the Utah Agricultural Experiment Station.

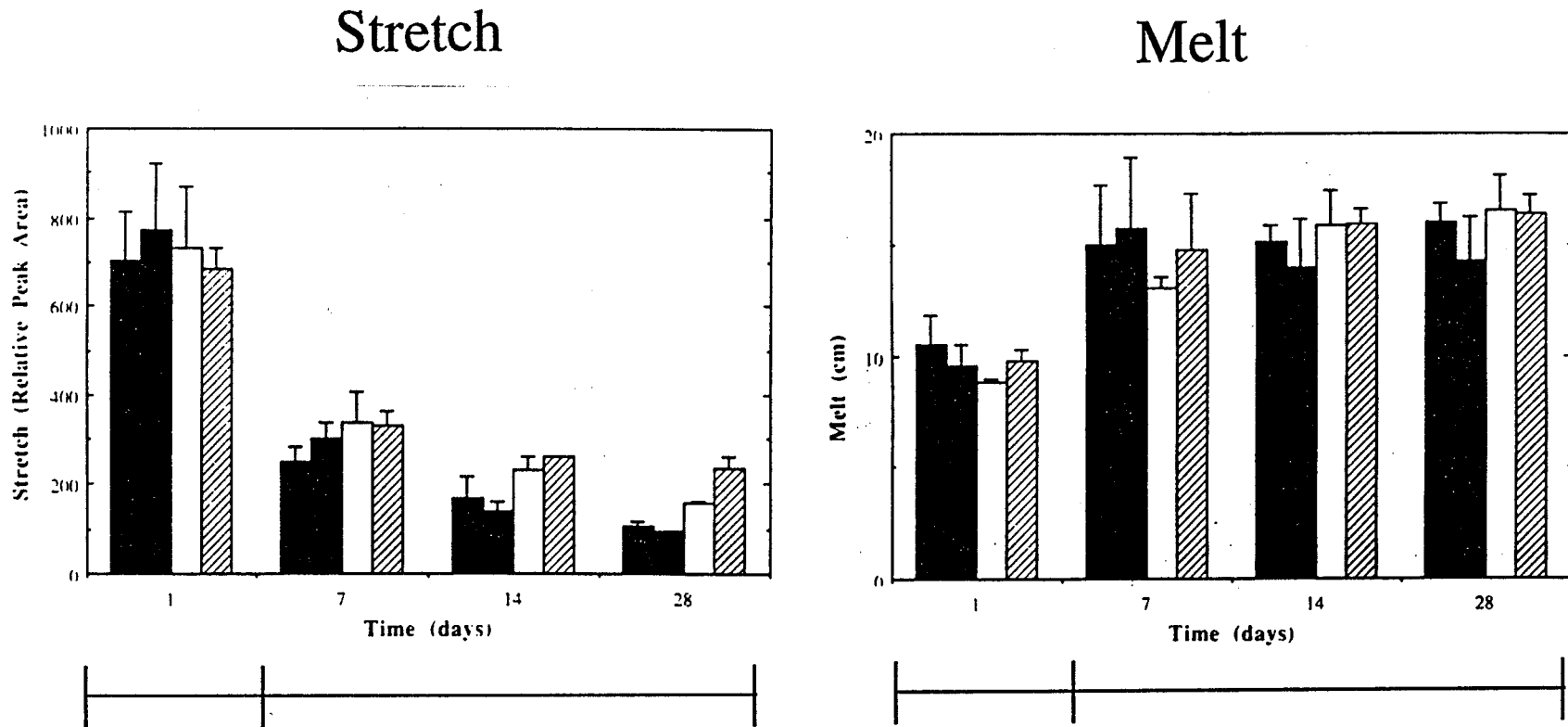
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12. Yun, J. J., D. M. Barbano, L. J. Kiley, and P. S. Kindstedt. 1995. Mozzarella Cheese. Impact of rod:coccus ratio on composition, proteolysis, and functional properties. *J. Dairy Sci.* 78:751.

Pizza Tests

- Fork Test - Cheese Industry Standard
- Helical Viscometer Test (Kindstedt)
- Tube Melt Test (Olson)
- Tensile Tests
 - Horizontal Extension (AK, 1993)
 - Vertical Extension (Apostolopoulos, 1994)
 - Vertical Extension (AK, 1995)
 - Squeezing Flow (AK, 1996)
 - Vertical Extension (Pena, 1996)
 - Vertical Extension (USU, 1996)

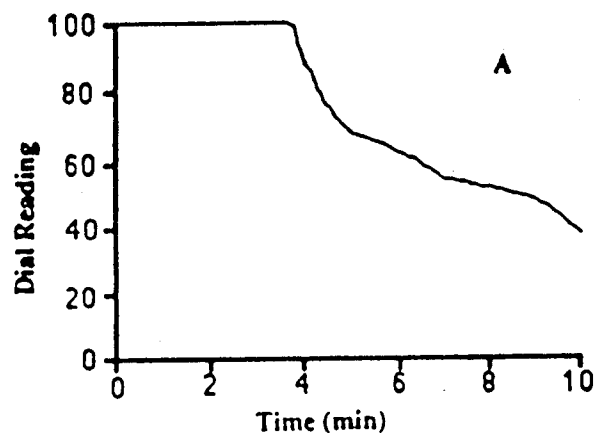
Inverse Relationship



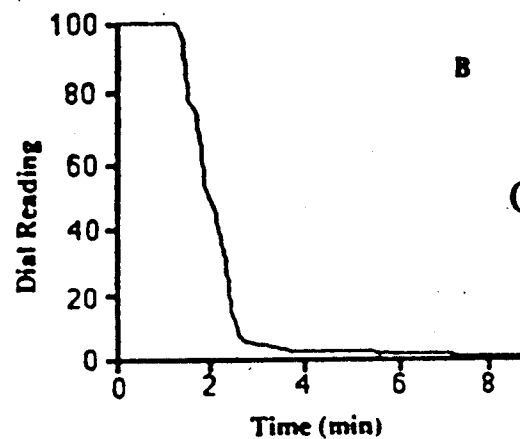
Oberg, C. J., R. K. Merrill, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of *Lactobacillus helveticus* culture on physical properties. *J. Dairy Sci.* 74:4101-4107.

Viscometer Profiles

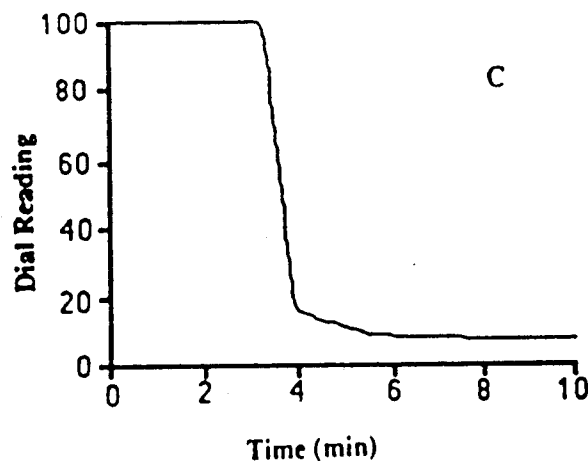
Mozzarella (0 d)



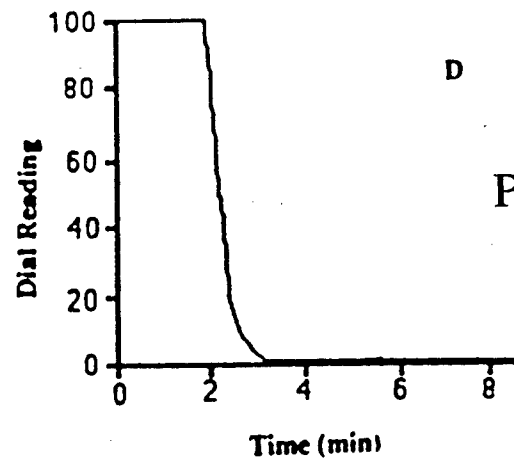
Cheddar (mild)



Mozzarella (28 d)



Processed Cheese



Oberg, C. J., A. Wang, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of proteolytic activity of thermolactic cultures on physical properties of Mozzarella cheese. *J. Dairy Sci.* 74:389-397.

Viscometer Profiles

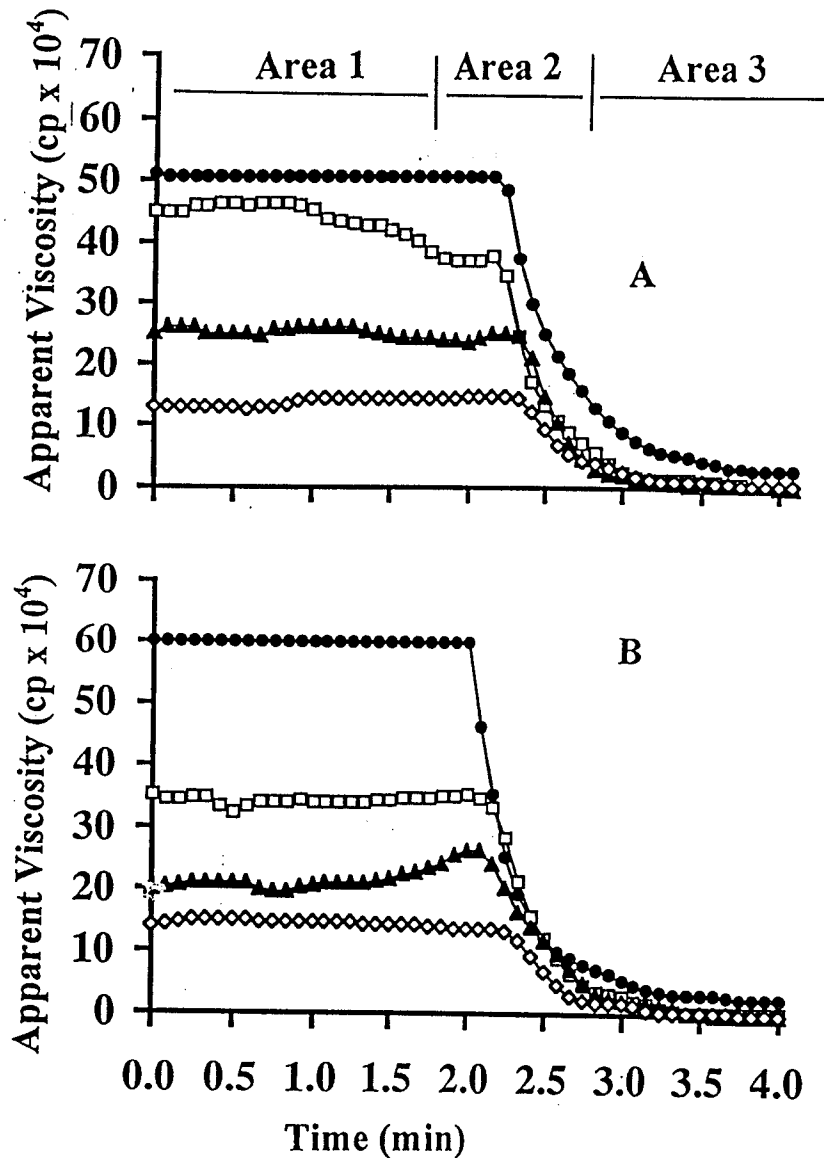
(day 1,7,14 and 28)

Part Skim Mozzarella

- Lower maximum viscosity

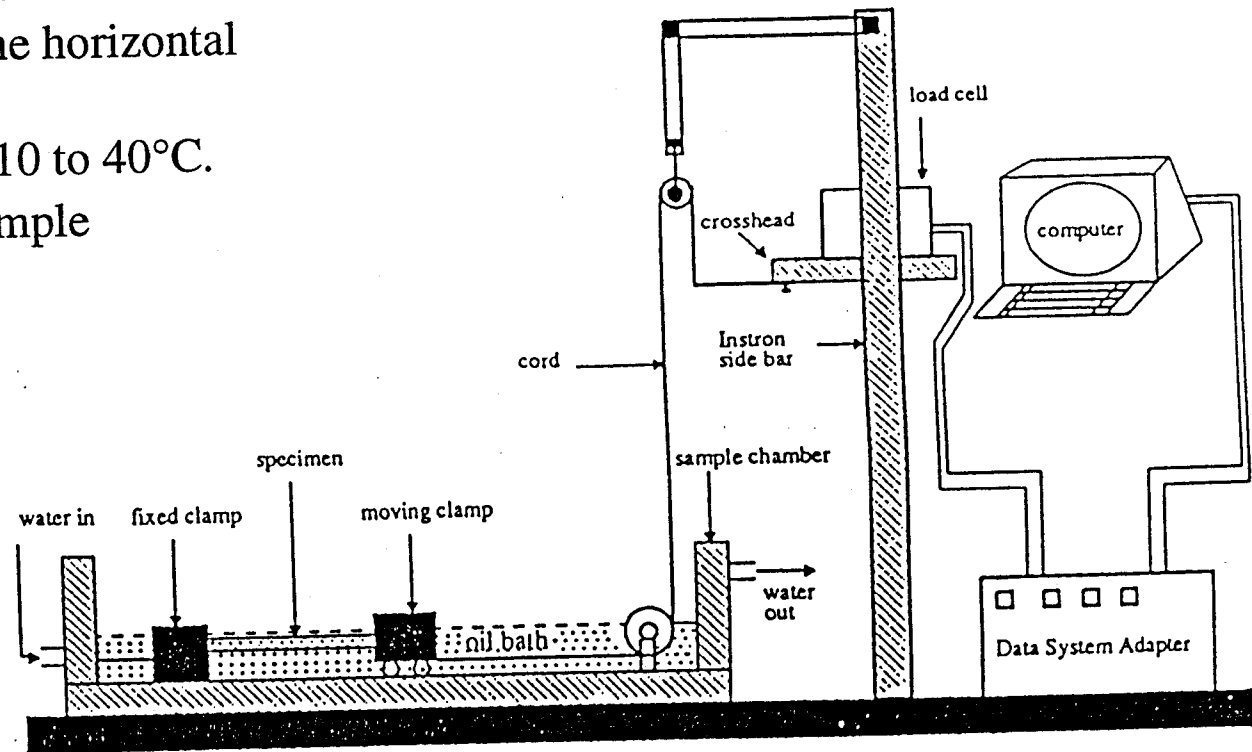
Low Fat Mozzarella

- Higher maximum viscosity
- Greater viscosity reduction in first 7 days



Uniaxial Horizontal Extension Apparatus

- Modified tensile test.
- Test performed in the horizontal plane.
- Temperature range 10 to 40°C.
- Oil bath controls sample temperature.

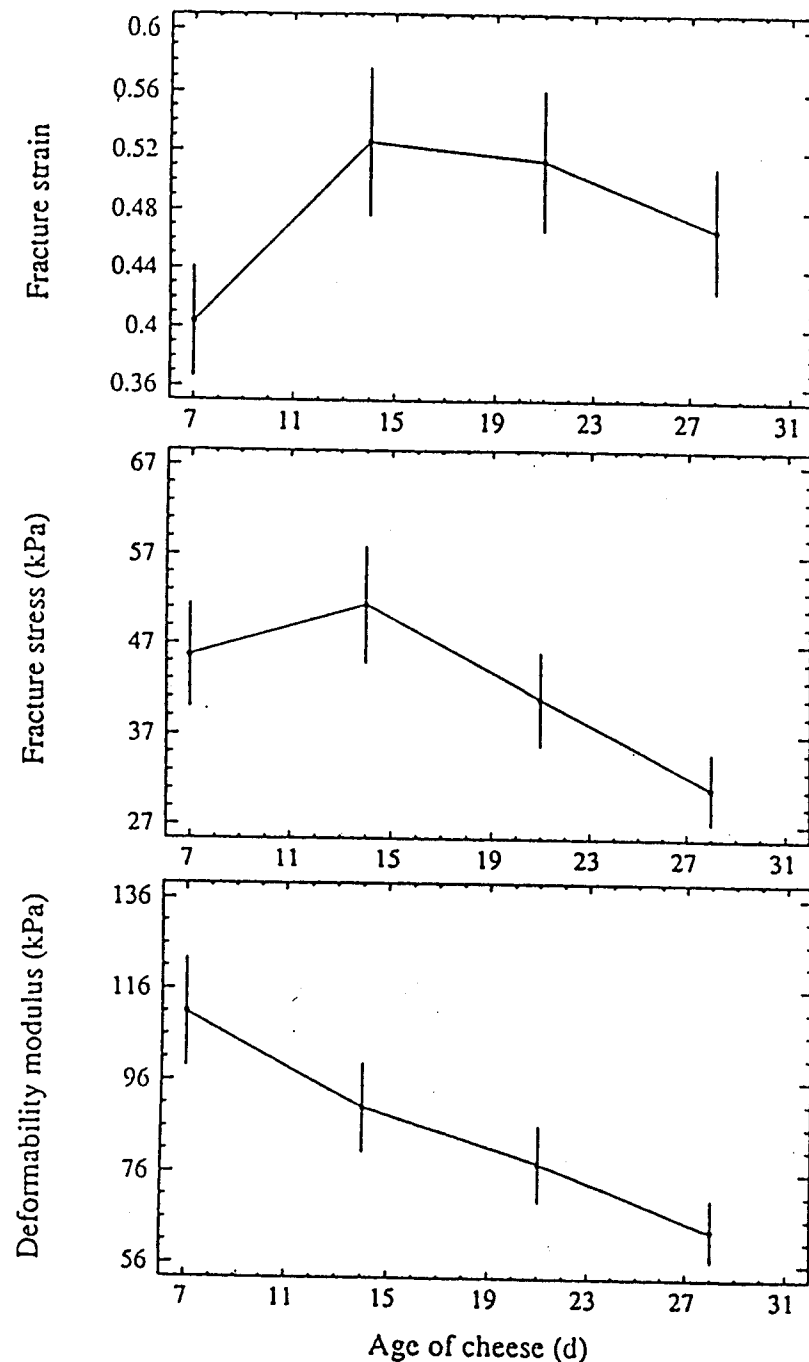


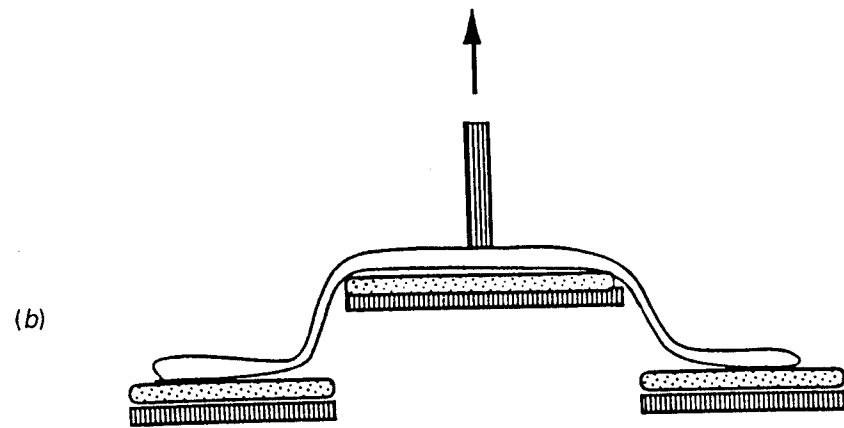
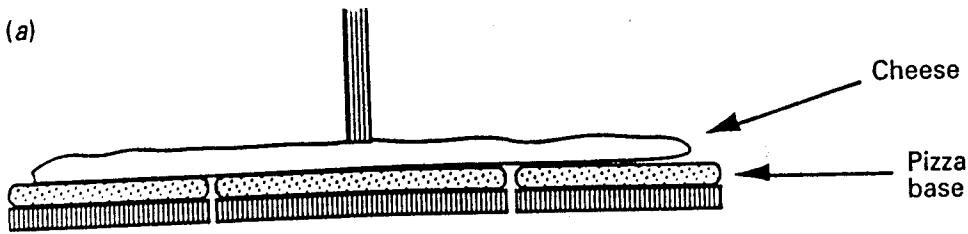
AK, M. M., D. Bogenrief, S. Gunasekaran, and N. F. Olson. 1993. Rheological evaluation of Mozzarella cheese by uniaxial horizontal extension. *J. Texture Studies* 24:437-453.

Uniaxial Horizontal Extension

- Fracture Strain Increased during first 2 wks
- Fracture Stress Decreased after first 2 wks
- Deformability modulus decreased over 4 wks

AK, M. M., D. Bogenrief, S. Gunasekaran, and N. F. Olson. 1993. Rheological evaluation of Mozzarella cheese by uniaxial horizontal extension. *J. Texture Studies* 24:437-453.

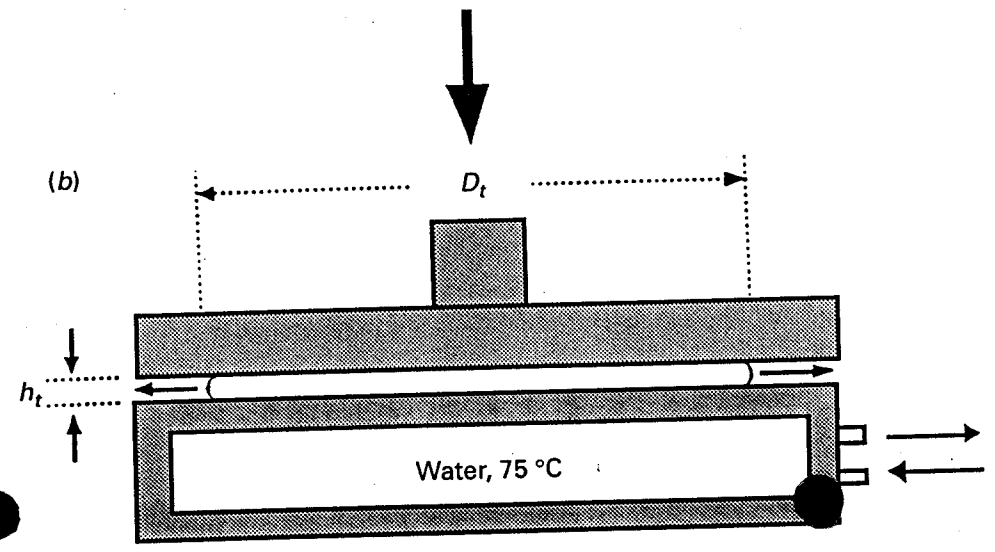
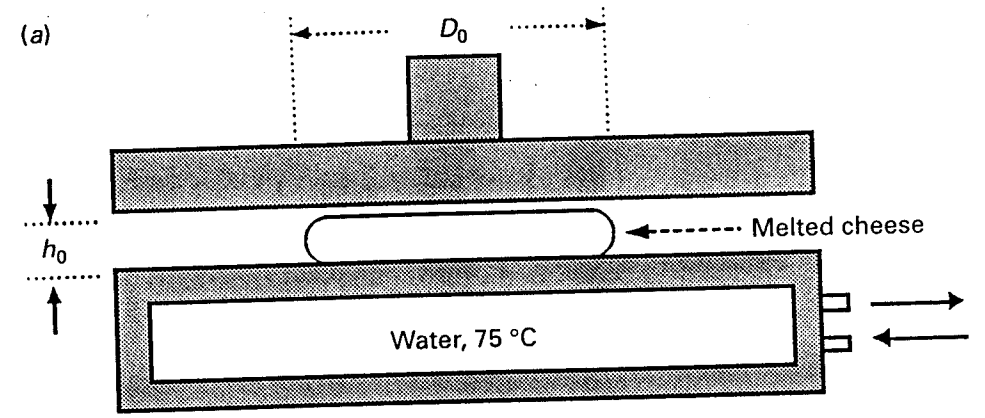




Vertical Extension Using a Pizza Base Template.

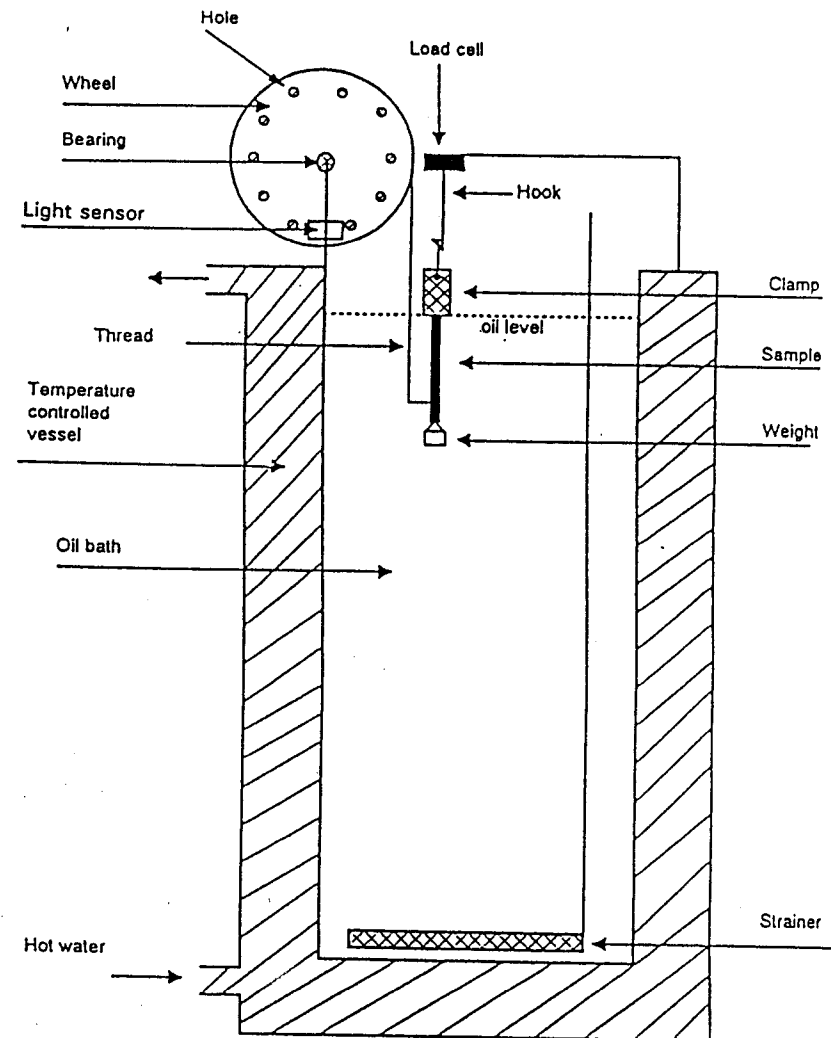
Compression Elongation

Apostolopoulos, C. 1994. Simple empirical and fundamental methods to determine objectively the stretchability of Mozzarella cheese. *J. Dairy Research* 61:405-413



Uniaxial Vertical Extension Apparatus

- Test performed in vertical plane.
- Temperature 60°C.
- Oil bath controls sample temperature.
- Elongation measured by an infrared emitter and detector.



AK, M. M., and S. Gunasekaran. 1995. Measuring elongational properties of Mozzarella cheese. *J. Textural Studies* 26;147-160.

Uniaxial Vertical Extension

- Temperature of the sample increases during test.
- Strain and stress rate is not constant during the test.
- Transient elongational (TE) viscosity decreased as the strain rate and sample temperature increased.
- TE viscosity was not able to measure the effects of proteolysis.

AK, M. M., and S. Gunasekaran. 1995. Measuring elongational properties of Mozzarella cheese. *J. Texture Studies* 26:147-160.

Squeezing Flow

Sample Preparation:

- Cylindrical samples cut parallel to the longitudinal axis of the cheese.
- Tempered to 30 to 60°C.
- Compressed from 5 to 50 mm/min.

Elongational Viscosity (Biaxial Stress Growth Coefficient)

- Decreases with temperature and cheese age.
- Is consistent with what is seen in the cheese industry.

AK, M. M., and S. Gunasekaran. 1995. Evaluating rheological properties of Mozzarella cheese by the squeezing flow method. *J. Textural Studies* 26:695-711.

Probe Type vs. Sensory Appraisals

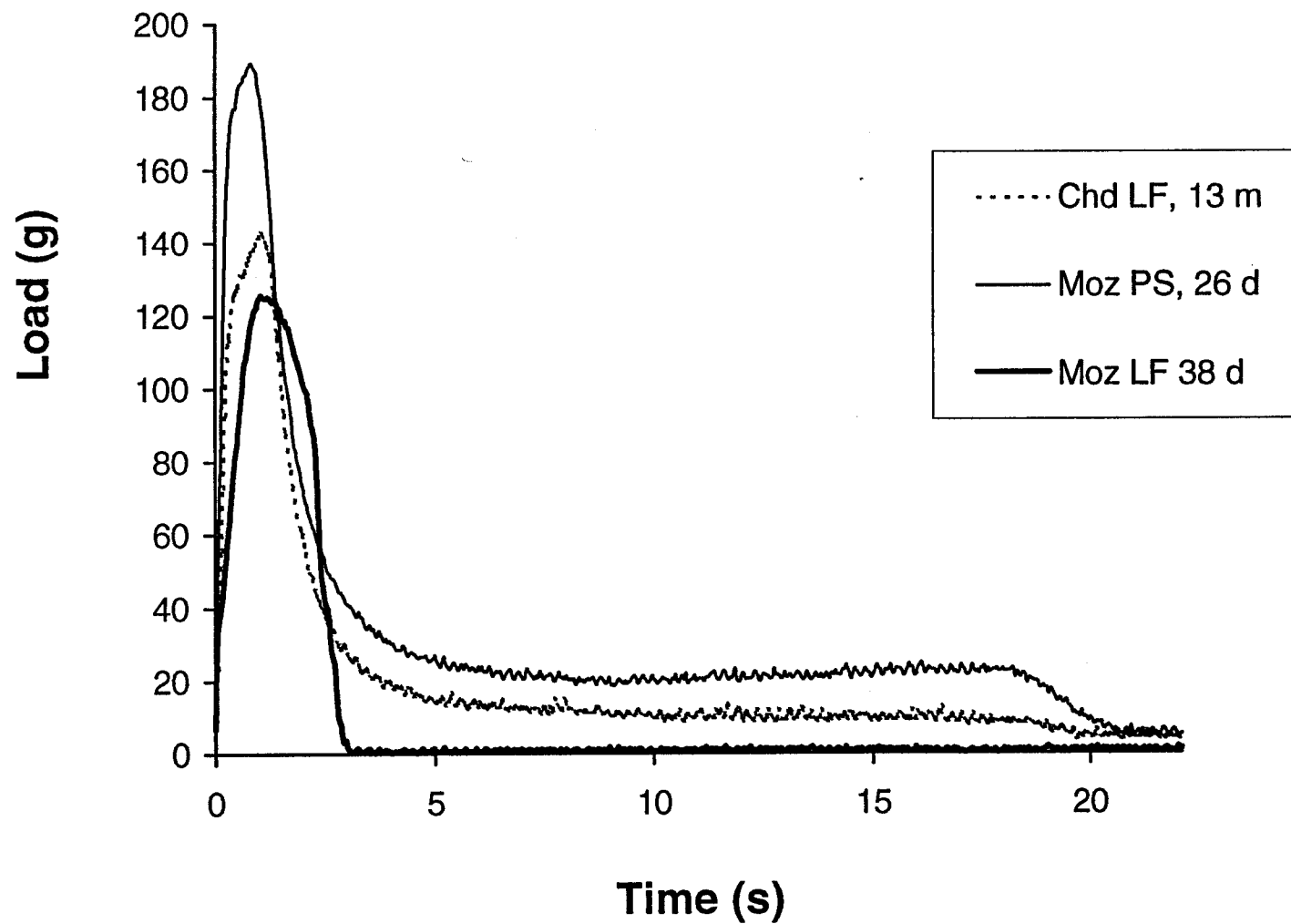
	Length (cm)	Width (cm)
Fork Shape	3.8	2.0
Solid Rectangle	3.2	0.8
Open Wire Rectangle	3.2	1.9

Conclusions:

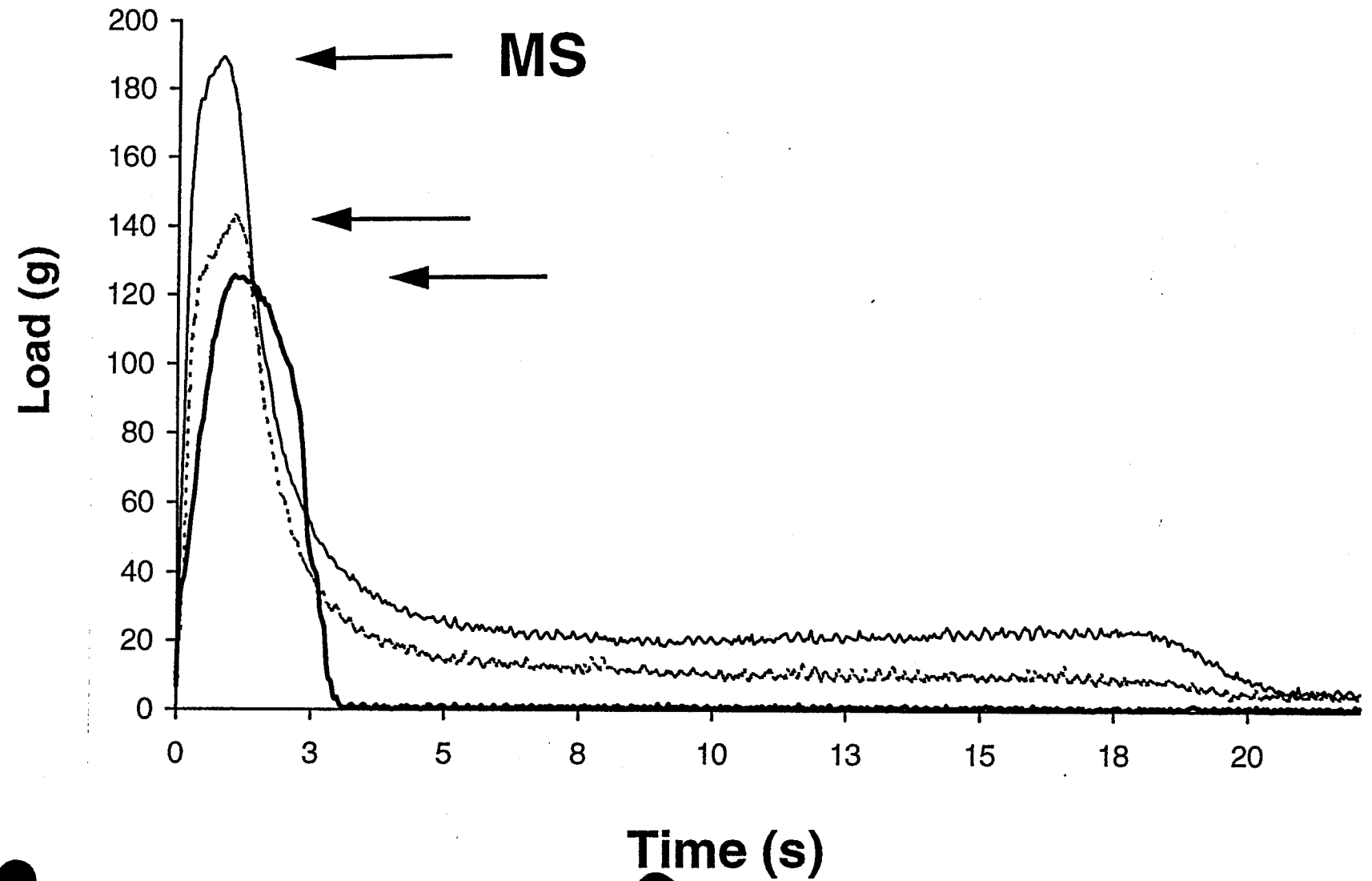
- Tension is a suitable means for measuring stretch.
- Open wire rectangular probe agreed with sensory evaluations.
- Method could be used in industrial setting.

Pena, J. L., A. Anzaldúa-Morales, G. Gastelum, and V. Nevarez. 1996. Poster paper presented at 1996 IFT Annual Meeting. New Orleans, LA.

Stretch Profile



Melt Strength



Stretch Length

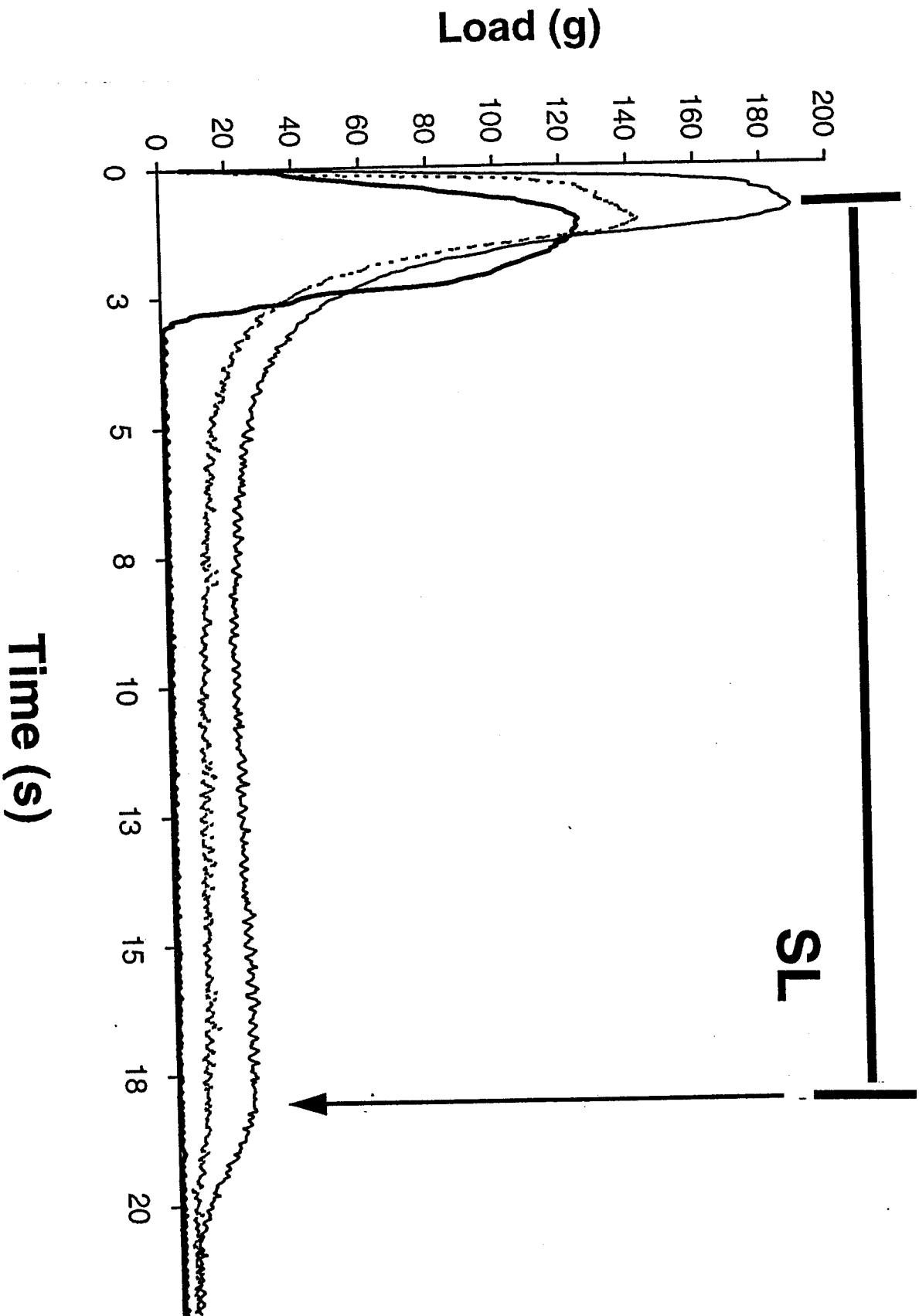


Figure 14

Stretch Quantity

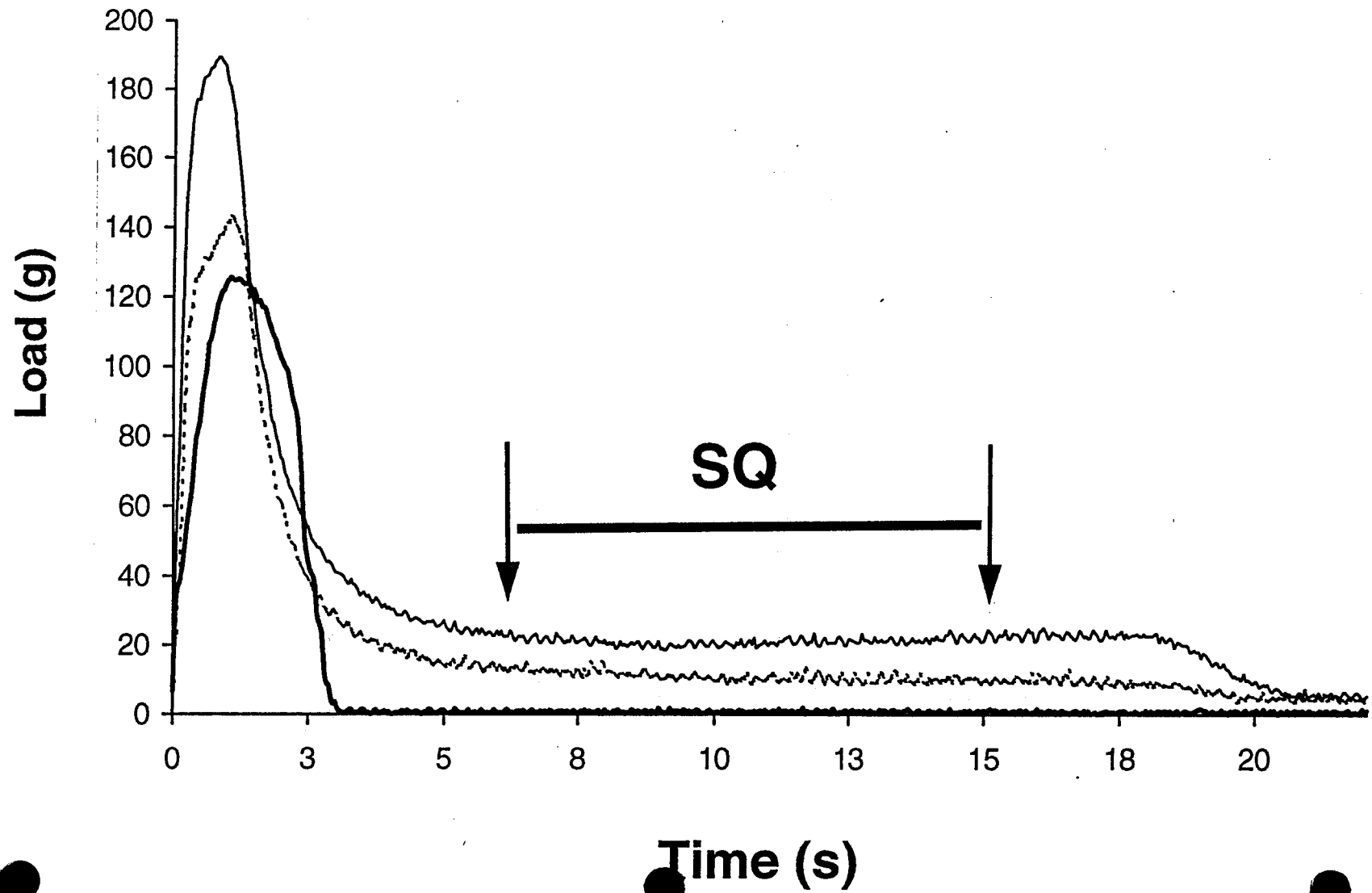


TABLE 1. Melt strength, stretch length, and stretch quality for low fat cheddar, part skim Mozzarella and low fat Mozzarella cheeses. Values generated from tail section analysis of tensile profiles.

CHEESE	AGE (d/m)	Melt Strength (g)	Stretch Length (cm)	Stretch Quantity
Ched LF	13 m	143	31	10
Moz PS	26 d	191	31	21
Moz LF	38 d	127	5	1