9-17-1997

Proceedings from the 34th Marschall Italian and Specialty Cheese Seminar

Various Authors

Proceedings from the 34th

MARSCHALL
ITALIAN & SPECIALTY CHEESE

SEMINAR

Presented by:

Rhône-Poulenc Dairy Ingredients

September 17 & 18, 1997
Dane County Expo Center
Madison, Wisconsin

Co-Sponsored by:
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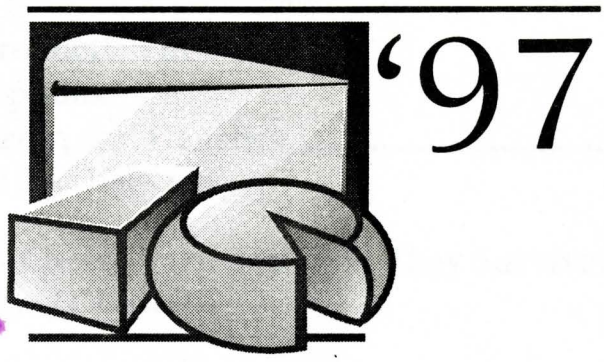
ITALIAN AND SPECIALTY CHEESE SEMINAR



The Italian Specialty Cheese Seminar is an event where cheesemakers, industry suppliers and technical experts come together in an atmosphere of camaraderie and shared interests; where educational forums and the latest technologies are presented; and industry related concerns are shared and solved through strong business relationships.

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Biology and Applications of Rod and Coccus Cultures

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1. Introduction

The temperatures employed in the manufacture of many Italian cheese varieties are higher than those used in the manufacture of cheese varieties such as Cheddar. Therefore, thermophilic lactic acid bacteria are used in the manufacture of these varieties. Additionally, the cultures typically contain both cocci and rods. The cocci present in these cultures is *Streptococcus thermophilus*. The rods which are present include *Lactobacillus delbrueckii* subsp. *bulgaricus* and/or *Lactobacillus helveticus*. The primary purpose of these cultures is to produce lactic acid from lactose, the principle carbohydrate present in milk. However, other culture characteristics which, depending on the application, may be important include galactose utilization, proteolytic activities, bacteriophage sensitivity, and production of exopolysaccharides.

2. General Characteristics

S. thermophilus is a gram-positive cocci which grows in chains. It ferments a limited number of carbohydrates (including lactose, sucrose, and glucose) and produces L(+)-lactic acid. It grows between 20 and 50°C and is generally considered among the most heat resistant streptococci (survives at least 30 min at 65°C).

L. delbrueckii subsp. *bulgaricus* and *L. helveticus* are gram-positive polymorphic rods which grow in chains. *L. delbrueckii* subsp. *bulgaricus* is more polymorphic than *L. helveticus* with the polymorphism being related to the age of the culture; short rods predominate in young cultures and longer filamentous forms occur more frequently in older cultures. Both lactobacilli ferment a limited number of carbohydrates; however an important distinction between the two species is that *L. helveticus* is capable of fermenting galactose while *L. delbrueckii* subsp. *bulgaricus* is incapable of fermenting this carbohydrate. Additionally, there is a significant difference in the mechanism by which these species utilize lactose, the mechanisms and significance of this difference will be covered in a subsequent sections. The genus *Lactobacillus* has been divided into three subgenera based on optimum growth temperature and fermentation end products. Both *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus* belong to the Thermobacterium subgenera based on their homofermentative metabolism and ability to grow at 45°C but not at 10°C. The isomer of lactic acid produced also differs between the two species. *L. helveticus* produces both L(+)- and D(-)-lactic acid, while *L. delbrueckii* subsp. *bulgaricus* produces only D(-)-lactic acid.

3. Protocooperative relationship

The relationship between *S. thermophilus and L. delbrueckii* subsp. *bulgaricus* or *L. helveticus* is referred to as protocooperative, as although these strains can grow in milk separately, they grow "better" as a mixed culture. When grown as a mixed culture in milk these organisms reach higher cell densities, grow faster, and produce more lactic acid at a faster rate. This relationship is believed to be due to *S. thermophilus* producing CO_2 and formic acid which stimulates the growth of the lactobacilli. The lactobacilli generate peptides from casein, the primary protein present in milk, which serve as a source of essential amino acids for *S. thermophilus*. The growth pattern of mixed cultures is characterized by *S. thermophilus* dominating early and the lactobacilli dominating latter, due to their ability to grow at lower pHs (Tamine and Robinson, 1985). In the manufacture of pasta filata type cheeses, the relatively short incubation time results in the *S. thermophilus culture dominating the fermentation.*

4. Lactose utilization

Lactose, a disaccharide composed of glucose and galactose, is the only free-form sugar present in milk (45-50 g/l). S. thermophilus, L. delbrueckii subsp. bulgaricus, and Lb. helveticus transport lactose via a lactose-galactose antiport system (permease) driven by an electrochemical proton gradient (Figure 1; Monnet et al., 1996). Lactose is cleaved by ß-galactosidase to yield glucose and galactose. The glucose moiety enters the glycolytic pathway with the fermentation end product being lactic acid. It is via this pathway that these organisms are able to derive the metabolic energy required for growth. The galactose moiety is excreted from the cells and accumulates in milk or cheese. Lb. helveticus differs from S. thermophilus and L. delbrueckii subsp. bulgaricus in that it is capable of transporting and metabolizing galactose. This occurs only after the lactose present in the growth medium is depleted. However, this will only occur if the culture is not inactivated by an heat treatment such as occurs in the manufacture of pasta filata cheeses or by storage at low temperatures (4-7^{oC}).

5. Proteolytic systems

Proteolytic systems in lactic acid bacteria contribute to their ability to grow in milk, and are necessary for the development of flavor in ripened cheeses. Lactic acid bacteria are amino acid auxotrophs typically requiring several amino acids for growth. The quantities of free amino acids present in milk are not sufficient to support the growth of these bacteria to high cell density; therefore, they require a proteolytic system capable of utilizing the peptides present in milk and hydrolyzing milk proteins (aS1-, aS2-, k-, and ß-caseins) to obtain essential amino acids. While growing in milk, lactic acid bacteria obtain essential amino acids in a variety of ways. They first utilize non-protein nitrogen sources such as free amino acids and small peptides. Casein, which composes 80% of all proteins present in milk, becomes the primary nitrogen source after non-protein nitrogen is depleted (Kunji et al., 1996). Proteolytic systems of lactic acid bacteria can be divided into three components, viz, enzymes outside of the cytoplasmic membrane, transport systems, and intracellular enzymes. A generalized schematic representation of the proteolytic system of lactic acid bacteria is presented in Figure 2. Extensive investigations have revealed that a cell-envelope associated proteinase is the only extracellular proteolytic enzyme present in lactic acid bacteria. A critical feature of the enzyme is its board cleavage specificity which results in the release of more than 100

oligopeptides from soluble ß-casein, 20% of which are small enough to be transported by the oligopeptide transport system. Transport of nitrogenous compounds across the cytoplasmic membrane takes place via group-specific amino acid transport systems, di/tri-peptide transport systems, and an oligopeptide transport system (Opp). Of these systems, Opp is of greatest importance during growth in milk. Once inside the cell, peptides are hydrolyzed by peptidases. Peptidase classes which have been identified include exopeptidases and endopeptidases. The greatest variety of enzymes are from the exopeptidase class which includes aminopeptidases, tripeptidases, and dipeptidases. No carboxypeptidases have been detected in thermophilic lactic acid bacteria. This combination of endopeptidases, aminopeptidases, tripeptidases, and dipeptidases converts the transported peptides into free amino acids required for growth.

The majority of S. thermophilus strains lack the cell-envelope associated proteinase while both L. delbrueckii subsp. bulgaricus and L. helveticus have complete proteolytic systems. The lack of a cell-envelope associated proteinases in S. thermophilus results in this organism being unable to obtain essential amino acids from intact caseins and, as previously discussed, partially explains the protocooperative relationship that exists between S. thermophilus and L. delbrueckii subsp. bulgaricus and L. helveticus. Additionally, strains of L. helveticus have been shown to typically have significantly greater general aminopeptidase activity (Sasaki et al., 1995). This characteristic, while not believed to provide any significant growth advantage, is thought to have a significant impact on cheese flavor development in ripened varieties.

6. Inhibitors

The primary inhibitors of rod and coccus cultures are antibiotics and bacteriophage. As a result of their use in treating mastitis, antibiotics can make their way into the milk supply. While advances in herd management and milk testing have greatly reduced their occurrence, antibiotics can still be responsible for inhibition of lactic starter cultures. In comparison to Lactococcus lactis, S. thermophilus and L. delbrueckii subsp. bulgaricus are more sensitive to penicillin and cloxacillin (Desmazeaud, 1996). However, bacteriophage currently are a more significant cause of inhibition of rod and coccus cultures. This is particularly true in the manufacture of Mozzarella cheese, where increased size of manufacturing facilities and strict manufacturing schedules has resulted in increased bacteriophage related problems. Phage infection of S. thermophilus is relatively common and results in a decrease in acid production and alters the rod:coccus ratio. The S. thermophilus bacteriophage characterized to date are all closely related. Bacteriophage which infect L. delbrueckii subsp. bulgaricus and L. helveticus have also been isolated, however their occurrence is believed to be relatively rare. Bacteriophage and bacteriophage resistance in S. thermophilusis currently receiving intense international research attention and will be covered in much greater detail by Dr. Sylvain Moineau in a subsequent presentation.

7. Starters and functional properties of Mozzarella cheese

Both the specific strains utilized and the ratio of rods to cocci can have a significant impact on the functional properties of Mozzarella cheese (Oberg and Broadbent, 1993). The functional properties of greatest interest are stretch, melt, and extent of browning. Attributes of the starter culture which are thought to influence these properties are rate of acid production, total proteolytic activity, and galactose utilization. Both the rate and extent of acid production is critical as it dictates the

moisture content in the cheese and the amount of calcium retained in the cheese matrix. The faster the rate of acid production and the lower the pH at draining, the lower the level of calcium retained in the cheese matrix. This has direct and significant effects on stretch and melt of the final product. Optimal stretch is obtained when the lowest pH reached is approximately 5.2, at both higher and lower values stretch is reduced. There is an inverse relationship between the level of calcium in the cheese matrix and melt, the lower the level of calcium the higher the melt. Manufacturing factors that can be manipulated include the level of inoculum, cooking, and cheddaring temperatures. The higher the inoculum level the faster the rate of acid production. The higher the cooking and cheddaring temperature the slower the rate of acid development. Culture selection can also play a critical role with strains of L. helveticus typically resulting in faster acid production. However, significant variation among strains of L. delbrueckii subsp. bulgaricus and L. helveticus exist, making strain selection a critical consideration.

The level of total proteolytic activity in the cheese is related to the final cell density and level of proteolytic activity of the rod, as the cocci is only weakly proteolytic. Hence the rod to coccus ratio is critical with the higher the ratio the greater the level of proteolytic activity in the cheese matrix. L. delbrueckii subsp. bulgaricus and L. helveticus both exhibit large strain-to-strain variation in proteinase activity, again making strain selection critical. Both stretch and melt are thought to be effected by the level of starter derived proteinase activity, however the rate and extent of acid production is believed to have greater impact on these attributes. Additionally, as the level of proteolytic activity in the cheese matrix increases, so will the level of amino groups available to participate in browning reactions. However, controlling browning is most easily accomplished by controlling the amount of galactose in the cheese matrix.

The amount of galactose in the cheese matrix has a significant impact on browning and the growth of nonstarter lactic acid bacteria during storage. Residual galactose participates in Maillard-browning reactions with free amino groups during cooking at high temperatures. Additionally, the residual galactose can serve as an energy source for growth of nonstarter lactic acid bacteria during storage. If the nonstarter flora includes heterofermentative organisms, rapid production of CO2 can result cracks in the cheese and swelling of packages. The use of galactose fermenting strains of S. thermophilus and/or L. helveticus reduces the level of galactose in the cheese matrix. However, the heat treatment that occurs during molding (52-66oC) and the relatively low temperature (4-7oC) of storage, limits the extent to which galactose fermenting thermophilic starters are able to metabolize residual galactose. Due to their higher metabolic activity at (4-7oC), lactococci are sometimes included in the starter to reduce the level of residual galactose.

8. Starters and flavor of hard grating Italian varieties

The mechanisms responsible for the formation of flavor compounds in hard grating Italian varieties (Parmesan and Romano) remains largely unknown. However, proteolysis is believed to be essential for flavor development in these varieties. Proteolysis in the cheese matrix is a sequential process involving milk-clotting enzymes, indigenous milk proteinases (particularly plasmin), enzymes from the starter culture and nonstarter lactic acid bacteria. The products are thought to serve as precursors for the generation of beneficial flavor compounds. Additionally, it is well established that the accumulation of hydrophobic peptides ranging in length from 3 to 27 amino residues results in the development of bitterness (Lemieux and Simard, 1992). It is widely believed that the level, specificity and rate of release of proteolytic enzymes from the starter culture play a central role in

determining which peptides and free amino acids accumulate in the ripening cheese. Therefore, strain selection plays a critical role in determining the flavor of the final product. While significant variation exists within species, in general, strains of L. helveticus have higher levels of intracellular peptidases than the other components of thermophilic starter cultures. This is particular true for general aminopeptidase activity, which is believed to be the enzyme primarily responsible for the hydrolysis of relatively short peptides and which has been demonstrated to be capable of hydrolysis of bitter peptides to nonbitter peptides and amino acids. The selection of strains to enhance flavor development in hard grating Italian varieties is currently an empirical process; however, as our understanding of the mechanisms responsible for production of beneficial and detrimental flavor compounds increases, it should become possible to select cultures which consistently produce high quality cheese on the basis of specific metabolic properties.

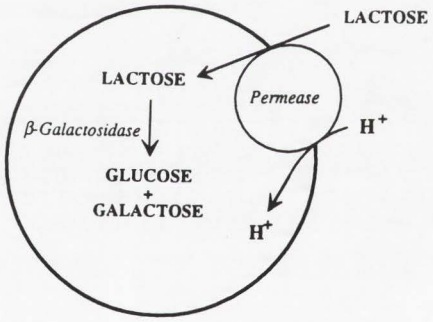

Figure 1. A schematic representation of the lactose/galactose antiport system which is utilized by Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, and Lactobacillus helveticus to transport lactose. Modified from Monnet et al. (1996).

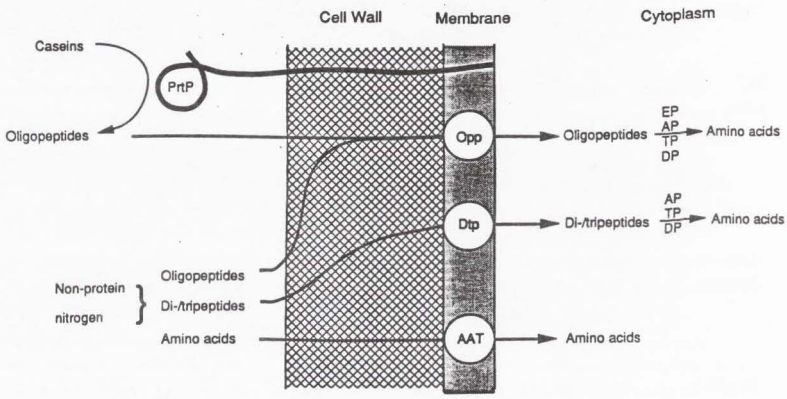

Figure 2. A generalized schematic representation of the proteolytic system of lactic acid bacteria. Abbreviations: PrtP, cell envelope-associated proteinase; Opp, oligopeptidase transport system; Dtp, di/tripeptide transport; AAT, amino acid transport systems; EP, endopeptidases; AP, aminopeptidases; TP, tripeptidases; DP, dipeptidases. Literature Cited

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Bacteriophages and Phage Resistance in *Streptococcus thermophilus*: An update

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Streptococcus thermophilus is a thermophilic lactic acid bacteria widely used as an industrial starter culture for the manufacture of yogurt and Italian cheeses. Last year was a record year in Canada for the production of yogurt and speciality cheeses and there is no indication these trends will change soon. It is a very well documented fact that increased productivity within existing facilities will lead to milk fermentation failures due to bacterial viruses, namely lytic bacteriophages. This natural phenomenon can cause substantial economic losses and is an ever-present financial threat. Due to their extensive growth, some yogurt and Mozzarella plants are now going through phage problems with *S. thermophilus*. This review will adress the recent advances in the biology *S. thermophilus* phages as well as in the development of phage-resistant strains.

Streptococcus thermophilus

Cells of this Gram-positive bacteria are spherical or ovoid (0.7 to 1.0 µm in diameter) and they are mainly found in pairs or long chains. *S. thermophilus* multiplies in the temperature range of 15 to 45°C but does not grow at pH 9.6. The growth is variable in 2% NaCl. *S. thermophilus* is facultatively anaerobe, chemoorganotrophic, catalase negative and its genome has a low G+C content. Lactic acid is produced only from few sugars including fructose, glucose, lactose, mannose, sucrose (Schleifer 1991). Their unique carbohydrate metabolism makes them easily identified by API50CH strips (Bio-Mérieux). Based on the 16S rRNA sequencing, *S. thermophilus* was phylogenetically included in the salivarius group with *S. salivarius* and *S. vestibularis* (Kawamura 1995). Specific DNA oligonulceotide probes have also been constructed for the rapid and accurate identification of *S. thermophilus* strains (Ehrmann 1992; Lick 1992, 1996). Only 20% of *S. thermophilus* strains contain (1 or 2) plasmids, so most strains cannot be differentiated by standard plasmid profile (Somkuti 1986, 1990; Mercenier 1994). Pulsed-Field Gel Electrophoresis (with *Sma*I) and ribotyping were showed quite reliable for differentiation of *S. thermophilus* strains (Salzano 1993, 1994; Roussel 1994, 1997).

Bacteriophages

A number of studies have described the general characteristics of *S. thermohilus* phages (Krusch, 1987; Prévots, 1989; Neve, 1989; Benbadis, 1990; Larbi, 1990; Carminati, 1992, Fayard, 1993; Brussow, 1994; Brüssow, 1995, Bruttin 1996, Le Marrec, 1997). The criteria examined included phage morphology (by electron microscopy), genome homology (by DNA-DNA hybridization), structural proteins (by SDS-PAGE), DNA restriction patterns (by digestion with endonucleases) and host range (by plaque assays).

To date, all *S. thermophilus* phages look alike! They have an isometric head (45 - 60 nm) and a long non-contractile tail (180 - 270 nm) which make them members of the *Siphoviridae* family (Ackermann 1987). Typically, *S. thermophilus* phages have a narrow host range. From a genetic stand point, the genome is made of double stranded DNA and restriction patterns indicate that genome sizes range from 34 to 44-kb (Le Marrec, 1997). It also appears that they all share DNA homology, albeit to different degrees (Mercenier 1994). In fact, *S. thermophilus* phages exhibit homology to each other in a modular fashion where homologous DNA regions are flanked by non-homologous sections (Stanley, 1997).

Recently, Le Marrec (1997) collected and analyzed 30 *S. thermophilus* phages from 7 different international collections. Interestingly, the phages could be divided in two groups based on number and size of the major (in high concentration) structural proteins and the DNA packaging mechanism. Nineteen phages (63%) had two major proteins (27 and 32 kDa) in their structure and the 11 other phages (37%) had three major structural proteins (15, 25, and 43 kDa). All phages with "two proteins" had cohesive genomic extremities (*cos* site) whereas the «three proteins» phages did not. These latter phages most likely packaged their DNA via a headful mechanism. Phages from these different proteins groups do share some DNA homology but to a much lesser extent then phages from the same protein group. Consequently, two species of *S. thermophilus* phages have been proposed. It seems that *S. thermophilus* phages could have originated from at least two ancestors which had different set of structural and replication proteins (Le Marrec, 1997). The biological significance of this classification is unknown but this phage diversity should be considered in the development of phage-resistant *S. thermophilus* strains.

Ecology

To establish a pratical prevention program, it is essential to identify the source of phages. Bruttin (1997) clearly showed that upon introduction of a new thermophilic starter cultures new phages were rapidly observed in the factory. The new phages were traced back to raw milk and not to a genetic recombination of existing resident phages. These recent findings point out raw milk as a natural resevoir of *S. thermophilus* phages. Since phages will always be present in dairy environs, our practical aim should be at controlling phages rather than trying to totally eliminate them.

The lytic cycle

Because phages are essentially genes wrapped into a protein coat, they cannot multiply without a host cell (Snyder 1997). To start the multiplication cycle (or lytic cycle), a phage adsorbs to a actively growing cell by binding to a specific receptor on the cell surface. These receptors are currently unknown in *S. thermophilus*. In *Lactococcus lactis*, the adsorption is a two-step process. In the first reversible step, the phage tail adsorbs to a carbohydrate component (rhamnose) of the cell wall and then, the phage is irreversibly anchored to a membrane protein (Valyasevi 1990, 1991). Data from the molecular characterization of *L. lactis* phages have shown that a phage protein (localize at the tip of the tail) has a calcium-binding site. This result confirms that some phage needs calcium to properly fold and attach to the starter culture (Schouler, 1994). *S. thermophilus* phages also need calcium to properly adsorb to the cells. These results emphasize the use of phosphate-containing medium (to sequestrate calcium) as an extra protection for propagating starter cultures.

After adsorption, the phage injects its DNA into cell and take control of the bacterial metabo-

lism. With the help of bacterial and phage enzymes, the phage DNA is replicated and many copies are accumulated within the cell. This step is quickly followed synthesis of phage proteins and the packaging of fully active progeny phages. Finally the cells break open (lyse) and numerous new phages are released and ready to infect other sensitive cells. Obviously the actual phage development is more complex than this basic process, proceeding through several intermediate stages in which the expression of different genes is exquisitely regulated by specific mechanisms (Snyder 1997).

The lytic cycle takes about 25 to 30 minutes for most *S. thermophilus* phages (Larbi 1990, Tremblay 1997). The number of phages released per cell depends on the phage and host but mainly range from 50 to 250 phage/cell (Larbi 1990, Tremblay 1997).

Lysogeny

In addition to the lytic cycle, some phages can undergo a lysogenic cycle. In this case, the (temperate) phage do not multiply but instead its DNA integrates into the host chromosome, replicating once every time the bacterial cell replicates (Snyder 1997). The state of lysogeny continues almost indefinitely until the temperate phage is induced (e.g. during damage of the host chromosome). After induction, the phage goes into the lytic mode and produce more phages which can lysogenize other cells. This phenomenon has been observed in *S.thermophilus* (Neve, 1989; Larbi, 1990; Carminati, 1992; Fayard, 1993; Brüssow, 1994, 1995). In theory, a starter culture could also be a source of phage within a plant. However, it appears to be very unlikely in *S. thermophilus* for two main reasons. First, very few strains of *S. thermophilus* have been shown to be lysogenic. Carminati (1992) found only one lysogenic strain out of 45 tested, Fayard (1993) found 12/120, Brussow (1994a) found 2/100 strains and Le Marrec (1997) found one out of the 51 strains tested. Secondly, testing of the lysogeny is part of the selection process for industrial strains. If found to contain temperate phages, the strain will be immediately discarded.

Interestingly, recent studies have showed that some *S. thermophilus* temperate phages do share DNA homologies with lytic phages (Fayard, 199; Stanley 1997). Bruttin (1997) showed that a site-specific deletion of a 2.4-kb DNA fragment from the temperate phage øSfi21 led to the generation of a new lytic phage. This discovery clearly indicates that temperate *S. thermophilus* phages can be a source of lytic phages in natural environments. However as mentioned above, the frequency is probably quite low in industry settings.

Phage detection

For many years, the inadequacy of existing plaque assays was a major hindrance to the developement of *S. thermophilus* phage research. These phages did not readily produce plaques in media commonly used in dairy laboratories. Recently, Le Marrec (1997) proposed the Elliker medium supplemented with beef extract (10 g/L) and ß-glycerophosphate (19 g/L) for many *S. thermophilus* phage/host systems. We also had some success with M17 supplemented with 0.3% non fat dry milk (Tremblay, 1997). These improved media should help in routing testing for *S. thermophilus* bacteriophages.

Another recent developement in the field of *S. thermophilus* phage detection was made by Brüssow's group from Nestlé (1994). A PCR-based method was developed for detection of *S. thermophilus* phages in cheese whey containing undefined starter cultures. PCR allowed the rapid

detection of 10^3 phages / ml of cheese whey. However, the authors justly added that PCR requires special equipments and skilled laboratory personnel. Thus it is not clear whether this method will find its way into the dairy industry (Brüssow 1994). Although the PCR method might not be used as a routine test, it could be very useful for the identification of phage groups or species within a sample that tested positive in standard quality control assays.

One simple stategy to control phage: the rotation

From a starter culture manufacturer standpoint, the obvious and successful way of dealing with phages is to rotate starters to avoid the development of specific phages. The rotation is based on the availability of a sufficient number of different and phage-unrelated strains. Difficulty in identifying truly phage-unrelated strains and non-uniform fermentations due to strain differences, are among problems associated with rotation schemes. From an evolutionary view, the use of different strains also favors the presence of a heterogenous phage population and the potential for recombination within the phage gene pool. In fact history has proven that despite a rotation system, virulent phages will eventually appear and build up within the plant.

It is reassuring to observe that many *S. thermophilus* phages have a narrow host range and propagate on a very limited number of different strains. However, the thought that most *S. thermophilus* phages share DNA homology is not a pleasant one! It is a well known fact that (homologous) recombination can occur between phages. Even more easily if there is DNA homology between them.

Phage-resistant strains

Developing a starter culture for a dairy fermentation is a relatively long process where strains have to be carefully selected based on numerous criteria. Thunnel (1986) listed 15 traits that needed to be characterized in a well-defined thermophilic starter. When the strains are selected after this long process, there is an obvious pressure to extensively use them. The quest for uniform products is another factor that favors the repeated use of performing strarter cultures. Unfortunately in dairy fermentations, the presence of phages and their evolving capabilities will prevent this long term use. In the last decade, extensive research has been conducted on interactions between phages and their hosts (Garvey 1995). Various techniques have been used to "save" the selected strains and "construct" phage-resistant derivatives. However most of the work has been done with the mesophilic starter *Lactococcus lactis*.

Bacteriophage-Insensitive Mutants (BIMs).

Historically, the development of BIMs has been (and still is) the method of choice for the construction of phage-resistant starter cultures. BIM is the term used to describe a spontaneous resistant bacteria which survives long exposure to a lytic phage. For most BIMs, the challenging phage can no longer adsorb to the cell, presumably due to mutations in the receptor. This theory still needs to be proven since little is known on the molecular mechanisms of the spontaneous mutations. Although, this technique has real advantages (simplicity and rapidity), it has also drawbacks. It can be short-lived as the mutation reverts to its original form. The resistance can be phage-specific as others phages can still attack the strain. Furthermore, some metabolic properties are lost or reduced in the process of generating BIMs. These modifications have a negative impact on the BIM growth

rate and on the industrial functionality. This is particularly true for *S. themophilus* where many BIMs can no longer grow in milk. Thus, exploiting natural evolutionary processes to develop phage-resistant derivatives is feasible but can be unpredictable.

Plasmid-coded phage defense barriers

In the last 15 years, significant progress in the genetics of *Lactococcus* has established the basis for constructing new phage-resistant strains through genetic modifications (Garvey 1995). *L. lactis* strains were found to possess many plasmids coding for natural defense mechanisms against phages. To date over 40 natural plasmids, conferring some degree of phage resistance, have been reported in the literature. The resistance systems are classified into 4 groups based on their general mode of action: adsorption blocking, penetration blocking, restriction/modification and abortive infection. In 1986, the first construction of a phage-resistant strain was reported, where such a plasmid was introduced into a industrial phage-sensitive *L. lactis* strain (Sanders 1986). The second generation of phage-resistant cultures was borned. Other studies have used a similar strategy with different plasmids and hosts (Garvey 1995; Emond 1997). Many «constructed» *L. lactis* strains are now extensively used in dairy countries.

A different situation is currently found in *S. thermophilus* for which very few phage resistance mechanism have been reported. One reason might be that only recently genetic techniques are available to manipulate *S. thermophilus* (Mollet 1993). The relative absence of plasmid might be another explanation for the scarcity of known phage resistance mechanisms in *S. thermophilus* (Moineau 1995). Nevertheless, four chromosomally encoded restriction/modification systems have been identified in *S. thermophilus*. The first endonuclease isolated was *Sst*134I and it is an isoschizomer of *Hpa*II (Solaiman 1990). The three other endonucleases isolated were *Sth*117I (Solaiman, 1991), *Sst*II (Benbadis, 1991) and *Sth*455I (Guimont 1993) and they are all isoschizomers of *Eco*RII. None of the genes have been cloned or sequenced, let alone used in commercial strains for improvement of phage resistance.

The rarity of plasmids and the chromosomal location of known-phage defense barriers in *S. thermophilus*, make it more difficult to develop food-grade strategies to improve phage resistance in this species (Moineau 1995b). A possible alternative is to introduce a phage resistance mechanism from other lactic acid bacteria, namely *Lactococcus*, into *S. thermophilus*. Recently, we reported the expression of a *L. lactis* R/M system in *S. thermophilus* (Moineau 1995ab). This genetic modification conferred strong resistance against phages of the «two» and «three» proteins groups (Moineau 1995b). Efficiency of plaquing ranging from 10^{-5} to 10^{-8} were observed. To date, this is the only published study on the construction of industrial phage-resistant *S. thermophilus* cultures based on the plasmid technology and a patent was filed on this application. A similar strategy was unsuccessful with a lactococcal abortive infection system (S. Moineau, unpublished).

Phage-based resistance mechanisms

Researchers are also turning to the phage genome as a potential source of resistance traits. If exploitation of phage genome as a source of inhibitory mechanisms is to attain maximum potential, understanding the phage lytic cycle at the molecular level is critical. Very few *S. thermophilus* phages have been studied in details. The complete nucleotide sequence of the first *S. thermophilus* phage (øO1205) was determined last year (Stanley 1997). This phage is a member of the «three

proteins» group. The availability of the entire DNA sequence will provide new opportunities for the development of novel anti-phage systems. Bruttin (1997) recently isolated a small DNA fragment from a temperate phage that confers slight resistance (EOP < 10^{-3}) against heterologous phages. This fragment encodes a gene for an unknown protein, although a phage repressor was suggested.

Other microbiological alternatives for phage controls

One possible alternative method to control bacteriophage is to replace *S. thermophilus* in starter blends with lactic acid bacteria from a different genus or species. *Pediococcus* strains, genetically-modified to ferment lactose, were proposed as potential replacement cocci for *S. thermophilus* (Caldwell 1996).

In some large dairy plants, the manufacture of different cheeses in rotation has been shown to successfully limit phage problems (S. Moineau, personal communication). A cheese diversification scheme allows the use of different type of starters which are diluting the population of specific phages.

Conclusions

Many *S. thermophilus* research programs are now in progress in many countries. These research programs will increase our basic and fundamental knowledge on the phages of the industrially important bacteria *Streptococcus thermophilus*. The first entire DNA sequence of a *S. thermophilus* phage will give critical information on its life cycle. These sequence results will allow the first comparisons between *S. thermophilus* and other LAB phages. The search for innate *S. thermophilus* phage defense systems will also be very active. In the next few years, these strategies should lead to the introduction of improved phage-resistant strains into the dairy market.

Acknowledgements

I would like to acknowledge the National Sciences and Engineering Research Council of Canada (NSERC - Operating) and the Fonds pour la Formation de Chercheurs et Aide à la Recherche (FCAR - Nouveau Chercheur) for funding our *Streptococcus thermophilus* research program. I also thank Denise Tremblay for her help during the preparartion of this manuscript.

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Exopolysaccharide Production in *Streptococcus thermophilus*: Physiology, Biochemistry, and Genetics.

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Introduction

Many strains of dairy lactic acid bacteria manufacture extracellular polysaccharides. These compounds may be produced as capsules which are tightly associated with the cell wall, or as a loose slime that is liberated into the medium. The term exopolysaccharide (EPS) is used to refer to both types of external polysaccharide (Sutherland, 1972). The EPS may be composed of one type of sugar monomer (homopolysaccharide) or consist of several types of monomers (heteropolysaccharide). Homopolysaccharides like dextran are produced by organisms such as *Leuconostoc mesenteroides*, while extracellular heteropolysaccharides are synthesized by several species of lactic acid bacteria including *Lactococcus lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* (henceforth referred to as *L. bulgaricus*) and *Streptococcus thermophilus*. Milk fermented with EPS-producing (EPS^+) lactic acid bacteria generally develops a ropy or viscous texture, and EPS^+ strains of *S. thermophilus* and *L. bulgaricus* are widely used in yogurt manufacture to enhance viscosity and reduce syneresis (Cerning, 1995). Research led by Dr. Don McMahon at Utah State University has recently shown that EPS^+ *S. thermophilus* can also be used to increase moisture retention in lowfat Mozzarella cheese (Low et al. 1998; Perry et al., 1997).

Physiology of EPS

In nature, bacterial exopolysaccharides are believed to fulfill diverse functions that involve cell protection, adhesion, cell-cell interactions, and other roles. The excellent water-binding properties of EPS, for example, may protect bacteria in low moisture environments, and capsular EPS can mask phage receptor sites and even inhibit amoebic attack or phagocytosis (Kang and Cottrell, 1979). The adhesive properties of EPS are of great scientific interest because of the key role it plays in the formation of biofilms and the pathogenesis of dental caries (Cerning, 1990; Whitfield and Keenleyside, 1995). Interestingly, one function that EPS production apparently does not provide is that of an energy reserve, since most EPS^+ bacteria are unable to catabolize the polymers they produce (Cerning, 1990).

In milk, EPS may impart viscosifying, stabilizing, and water-binding functions (Van den Berg et al., 1995). Many dairy processors rely on EPS^+ starters to control syneresis in yogurt, and this practice is particularly widespread in countries where the use of stabilizers is prohibited (Cerning, 1995). Although other factors can be involved, the influence of EPS on the viscosity of fermented milk is primarily determined by the amount of polymer produced. Reports suggest that the amount of EPS produced by *S. thermophilus* in milk ranges from 50 to 300 mg per liter, depending on the particular strain and growth conditions (Cerning, 1995). EPS yield is not a direct function of cell growth. Instead, production is almost always enhanced by growth at lower incubation temperatures (Cerning, 1995; Mozzi et al., 1995). EPS production may also be favored at near neutral

pH and by a high carbon:nitrogen ratio in the growth medium (Gancel and Novel, 1994; Sutherland, 1972).

EPS Structure and Biochemistry

As a result of the use of different growth media, bacterial strains, and EPS isolation and purification methods, conflicting reports exist regarding the sugar composition of the EPS, as well as the amount of EPS produced by individual lactic acid bacteria. Despite these discrepancies, most reports indicate that *S. thermophilus* EPS is composed primarily of galactose, glucose, and rhamnose (Figure 1).

The biosynthesis of heteropolysaccharides by dairy lactic acid bacteria has not been extensively investigated, but most researchers believe that the mechanism is similar to that proposed for EPS production in Gram-negative bacteria because both

Figure 1. Structures of *Streptococcus thermophilus* heteropolysaccharides.

S. thermophilus CNCMI 733 and Sfi6 (Doco et al., 1990; Stingele et al., 1996):

a-D-Gal (1 → 6)

→ 3) b-D-Gal (1 → 3) b-D-Glc (1 → 3) a-D-GalNAc (1 →

S. thermophilus OR 901 (Bubb et al., 1997):

b-D-Gal (1 →6) b-D-Gal (1 → 4)

→ 2) a-D-Gal (1 → 3) a-D-Gal (1 → 3) a-D-Gal (1 → 3) a-L-Rha (1 → 2) a-L-Rha (1 →

S. thermophilus MR-1C (Low et al., 1998):

Gal (1 → 6) Gal (1 →4)

Fucose (1 → 3)

→ 2) Gal (1 → 3) Gal (1 → 3) Gal (1 → 3) Rha (1 → 2) Rha (1 →

Abbreviations: Gal = galactose; GalNAc = *N*-acetyl-D-galactosamine; Glc = glucose; Rha = rhamnose.

EPS appears to be independent of the carbon and energy source used to support bacterial growth (Sutherland, 1972). Once the repeating unit has been assembled, the lipid-linked intermediates are translocated across the membrane and polymerized on the outside of the cell (Whitfield and Keenleyside, 1995).

Protein homology studies of the *S. thermophilus* EPS gene products suggest that enzymes specific for EPS biosynthesis in this bacterium include glycosyltransferases needed for the assembly of repeating units as well as enzymes involved in chain length determination, export/polymerization functions, and production of sugar nucleotide precursors for monomers (e.g. *N*-acetyl-D-galactosamine) that are unique to the EPS (Stingele et al., 1996). Enzymes whose function is not unique to EPS production include those that are involved in the synthesis of sugar nucleotide precursors. In *E. coli*, one example in the latter category is GalE (UDP-glucose 4-epimerase), an enzyme that is needed for the production of uridine diphospho (UDP)-galactose, but whose "normal" role involves the interconversion of UDP-galactose and UDP-glucose in the Leloir pathway for galactose catabolism (Whitfield and Keenleyside, 1995). *S. thermophilus* strains typically are unable to ferment galactose, yet genes encoding GalE and other Leloir enzymes have been isolated from this bacterium (De Vos and Vaughan, 1994). This finding has led to suggestions that in *S. thermophilus*, GalE and perhaps GalT (UDP glucose-hexose-1-phosphate uridyl transferase) may still function in EPS production (Poolman, 1993).

The involvement of an isoprenoid glycosyl carrier lipid in EPS production is particularly important because it is the same lipid carrier used by bacteria for the synthesis of peptidoglycan, lipopolysaccharide, and techoic acids (Cerning, 1990). As a consequence, competition for isoprenoid lipid carrier may be the most important limiting factor in EPS biosynthesis (Sutherland, 1972). This hypothesis is supported by observations that incubation of bacteria under conditions which stimulate cell growth and division reduce EPS production, while growth conditions which reduce the demand for new cell wall synthesis (such as incubation at a suboptimal temperature) tend to increase EPS production (Sutherland, 1972; Cerning, 1995).

Genetics of EPS

In many thermophilic and mesophilic lactic acid bacteria, the EPS^+ phenotype is unstable and may be permanently lost following repeated cell transfer or after prolonged incubation (Cerning, 1990). The instability of EPS^+ is a problem in industry because EPS^+ bacteria must be occasionally reselected from stock cultures to maintain this attribute (Stingele et al., 1996). In mesophilic lactic acid bacteria (e.g. *Lactococcus lactis*) EPS^+ is often encoded by plasmid DNA (Cerning, 1990; De Vos, 1996), which provides a simple genetic explanation for the instability of EPS production in these strains, i.e. plasmid loss. In contrast, genes for EPS production in thermophilic lactic acid bacteria such as *S. thermophilus* and *L. bulgaricus* are thought to be chromosomally encoded (Stingele et al., 1996). Thus, the unstable nature of these genes is not understood, but may be related to mobile genetic elements or genomic instability.

The *eps* gene cluster of *S. thermophilus* Sfi6 was recently characterized by Stingele and coworkers (1996), who showed EPS synthesis involved 13 genes, *epsA-M* , that are sequentially arranged on a 14 kilobase pair fragment of the *S. thermophilus* chromosome. Interestingly, experiments using the polymerase chain reaction showed *epsA*, B, C, and *D* genes may also be present in EPS^- strains of *S. thermophilus*, but all of the other genes (*epsE-M*) were only detected in EPS^+ strains. Protein homology studies using deduced amino acid sequences from each gene suggested *epsA* may encode a protein involved in the regulation of EPS expression. The authors were not able to ascribe any function to EpsB, L, or M, but homology studies suggest *epsC, D, J, and K* may encode enzymes involved in polymerization and export. That work also showed that *epsE, F, G,* and *I* genes likely encode the glycosyltransferases that are responsible for assembly of the repeating unit. In addition, the EpsE protein showed significant homology with galactosyltransferase enzymes from several bacteria. This enzyme is known to catalyze the first step in the synthesis of the repeating unit: transfer of galactose-1-P from UDP-galactose to the undecaprenyl-phosphate carrier lipid.

Finally, the product of *epsH* showed homology to acetyltransferases, which may be involved in the synthesis of sugar nucleotide precursors for *N*-acetyl-D-galactosamine (Stingele et al., 1996).

Applications for EPS in Italian Cheese

Recent growth in the market for reduced-fat foods has raised interest in the development and manufacture of reduced-fat and low-fat Mozzarella cheese. Unfortunately, fat removal has undesirable effects on the physical properties of Mozzarella (McMahon et al., 1993). Specifically, the cheese becomes tough and rubbery, more heat is required for melt, and the cheese loses pliability rapidly upon cooling (Mistry and Anderson, 1993). Studies of Mozzarella cheese at Utah State University have revealed that these properties are influenced by the moisture level in the cheese (Merrill et al., 1994). Analysis of cheese microstructure showed that in full fat or part-skim Mozzarella, a large portion of the water in the cheese is contained in channels that are formed in the protein matrix by trapped fat globules (McMahon et al., 1993, Oberg et al., 1993). In low-fat Mozzarella cheese, however, there are very few fat globules to break up the protein strands so these channels become more narrow. This creates less space for water in the cheese matrix and results in cheese with a lower moisture level (Oberg et al., 1993) and, as a consequence, a tough, rubbery texture and poor melt and stretch properties (Merrill et al., 1994).

Make procedures for reduced-fat and low-fat Mozzarella cheese can be modified to increase moisture levels and improve the body, texture and functional properties of these products. Merrill and coworkers (1994) found that elevated pasteurization temperature, milk pre-acidification, larger cutting knives, and lower cook temperatures facilitated moisture retention in reduced-fat Mozzarella (Merrill et al., 1994). Subsequent work by Perry et al. (1997) indicated that an EPS^+ starter pair, *S. thermophilus* MR-1C and *L. bulgaricus* MR-1R, could also be used to increase moisture retention in low-fat Mozzarella cheese. To further investigate this observation, low-fat Mozzarella cheese was manufactured by Low et al. (1998) using different combinations of EPS^+ and EPS^- starters. The EPS^+ starters included in that work were *S. thermophilus* MR-1C and *L. bulgaricus* MR-1R, while EPS^- starters were *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100. The cheese was made using combinations of one rod, a *Lactobacillus* species and one coccus, a *S. thermophilus* strain, as starter pairs. Ten kg vats of low-fat Mozzarella cheese were made with four starter combinations: MR-1C plus MR-1R, MR-1C and LH100, TA061 and LH100, and TA061 plus MR-1R.

After one day of storage at 4°C, grated samples of the cheeses were analyzed for moisture content using a vacuum oven. This analysis revealed that the MR-1C + MR-1R cheese was 61.9% water, the MR-1C + LH100 cheese had 61.6% moisture, and both the TA061 + LH100 cheese and the TA061 + MR-1R cheese contained 60.0% moisture. Analysis of variance (ANOVA) of this data showed that the only factor which significantly influenced the moisture in the cheeses was the coccus used in the cheese making ($P = 0.001$).

To determine whether the *S. thermophilus* MR-1C EPS was responsible for the water-binding property of MR-1C, we decided to inactivate the EPS producing ability of MR-1C using a gene replacement strategy. Partial characterization of the *eps* gene cluster of *S. thermophilus* MR-1C showed that it was similar in organization to that of Sfi6 at least through *epsF*. Deduced amino acid sequences from the EpsA-F region of MR-1C indicated that these proteins were between 95 and 99% identical with those in Sfi6. Because Stingele et al. (1996) showed that *eps A-D* genes may be present in EPS^- strains of *S. thermophilus*, we decided to target *epsE*. A 2.4 kilobase pair region containing a fragment of the MR-1C *epsE* genes was obtained by polymerase chain reaction and cloned into *E. coli*. An internal deletion was created in the cloned *eps E* fragment and then an integration vector containing the partially deleted *epsE* gene was constructed in pSA3, a temperature sensitive plasmid which encodes erythromycin resistance (Bhowmik et al., 1993; Dao and Ferretti,

1985). The recombinant plasmid was introduced into MR-1C by electroporation, and integration of the plasmid into the host chromosome was induced by incubation at a nonpermissive temperature (45°C) in the presence of erythromycin. After several transfers at 45°C, the cells were incubated without erythromycin at 37°C, a temperature which is permissive for pSA3 replication, to induce a second DNA recombination event that leads to either replacement of the wild type gene by the deleted form of the gene or reversion to the wild-type (Bhowmik et al., 1993). After the gene replacement experiment was complete, an EPS$^-$ strain was isolated by selection on milk agar which contained ruthenium red dye (Stingele et al., 1996), and loss of the capsule was confirmed through the use of the Duguid capsule staining method (Duguid, 1951). DNA sequence analysis of the EPS$^-$ mutant, which was designated *S. thermophilus* DM10, showed that this bacterium contained a frameshift mutation in the *epsE* gene (Low et al., 1998).

Since our objective was to establish whether the MR-1C EPS was responsible for increased moisture retention in low-fat Mozzarella cheese, low-fat Mozzarella cheese was made with the EPS$^-$ strain *L. helveticus* LH100 and either *S. thermophilus* MR-1C or the EPS$^-$ mutant DM10. Low-fat Mozzarella cheese made with DM10 and LH100 contained 58.5% moisture, while that made with MR-1C + LH100 contained 61.9% moisture. ANOVA confirmed that this difference was significant ($P = 0.007$).

To learn more about the water-binding property of the MR-1C EPS, we have also begun to investigate its structure. As shown in Figure 1, the MR-1C EPS has a novel octasaccharide repeating unit composed of galactose, rhamnose, and fucose in a ratio of 5:2:1. In summary, we have shown that *S. thermophilus* MR-1C significantly increased moisture retention in low-fat Mozzarella cheese. Experiments with the EPS$^-$ mutant strain DM10 showed that this effect was due to the capsular EPS produced by MR-1C, and structural studies indicate that the MR-1C EPS is unlike any polymer previously described in dairy lactic acid bacteria.

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Traditional European Cheese Varieties: Will They Survive?

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A summary of the regulatory environment and the status of key European regulations that impact the production of specialty cheeses will be discussed. The Protected Denomination of Origin (**PDO**) designation for individual European specialty cheeses will be explained and an overview of the PDO cheeses of Europe will be presented. Special emphasis will be given to the main technological aspects of the traditional cheese making processes and to specific characteristics of unique cheese varieties.

The European Union (**EU**) strategy and justification for protecting unique local cheese varieties will be explained with respect to the environmental and sociological importance of maintaining populations in areas at risk for depopulation and areas with specific environmental problems. Some specialty cheese varieties are on the verge of extinction. Loss of a cheese variety can be viewed as a loss of part of the cultural identity of a region.

In addition to the historical and social significance of producing traditional cheeses, their economic importance in the European market will be discussed. The commercial strategy used to market traditional cheeses, such as use of special identifications like **Farm Cheese** in England, **Boerenkaas** in Holland, **Fromage fermier** in France, **Queso artesano** in Spain, and **Formaggio artigianale** in Italy will be reported. Most European traditional cheese are from the Mediterranean area and usually are characterized by production on small farms, in small quantity per day, with unique quality, and high variability. The consumer accepts some variability in quality and quantity of locally produced specialty cheeses, but this variability represents a limiting factor with respect to commercial development.

Will traditional European cheese varieties survive? **YES!** They are part of the fabric of life, history, and culture in various regions of Europe and most specialty cheese varieties will be preserved. The challenge is to help the farmers improve cheese quality by reducing the range of variation in cheese quality. This can be done by application of modern knowledge of cheese making and food safety to produce unique, safe, and more consistent products using appropriate technologies that preserve the tradition of these unique cheese varieties.

Importance of PDO Specialty Cheese in the EU

The European community has defined the concept Protected Denomination of Origin (PDO) for products in Regulation EEC No. 2081/92. The PDO designation from the European community indicates a product whose qualities derive exclusively from a geographic area, including human and

number of PDO cheeses produced in each country and their percentage of the total cheese production within each country are shown in Table 1. France and Italy each produce about 30 different PDO varieties of cheese, while Greece produces at 20 different PDO cheeses. Spain, the United Kingdom, and Portugal produce about 10 different PDO cheese each. However, the number of PDO cheeses produced in a country is not necessarily directly related to the economic importance of PDO cheeses for that country. For example, Italy produces 30 different PDO cheese varieties and these varieties accounted for 49% of total cheese production in Italy. Greece produces 20 different PDO cheese varieties that account for 42.9% of the total cheese production in Greece. In contrast, France produces 33 different PDO cheese varieties and these varieties only represented 10.4% of the total cheese production in France, while in Spain produces 11 PDO cheese varieties that account for 4.5% of the total cheese production in Spain. Although the economic impact of production PDO cheeses in France and Spain is small at a national level compared to Italy and Greece, their contribution to the economy and culture of local rural areas in France and Spain is important. The top four PDO cheese varieties for Italy, France, Spain, and Greece are shown in Table 2.

In total there are over 1000 different cheese varieties produced in the European Union countries. The PDO cheeses are only a small fraction of the total varieties of cheese. Thus, there are many additional important cheese varieties that may satisfy the criteria for PDO. As of 1995, 118 of these many cheese varieties produced in Europe have been brought forward by people from specific geographic regions for characterization, definition, and designation as very unique products that merit the designation of PDO.

Once a cheese variety receives the PDO designation, the producers of that cheese are required to form a consortium to establish quality standards and a process for verification of compliance by the cheese producers with the quality standards. The standards can be based on conformance to a defined manufacturing procedure, product composition, and other methods of quality evaluation. Cheese made by each producer will be evaluated periodically and if acceptable, the cheese will receive the PDO certification and be stamped with the PDO insignia for that cheese variety. If the cheese does not comply with the quality standards of the consortium, it can be marketed by the producer as the same cheese variety without the PDO designation, but it will be sold at a lower price in the market place.

Characteristics in support of PDO designation

There are many characteristics of a cheese variety that are considered in the application for the PDO designation by the European Union. To be designated PDO by the European Union, a product must have "qualities derived exclusively from a geographic area, including human and natural factors and whose production, processing, and manufacture take place in a defined geographic area". Some factors that are considered are:

1. contribution of natural pasture of the region to flavor,
   examples: Pecorino Toscano - Italy
   Castelmagno - Italy

2. the breed of milk producing animal,
   example: Le Beaufort - breed Tarentaise - France

3. use of raw milk,
   example: Vastedda - Sicily, Italy

4. unique characteristics of rennet used,
   example: Queso de la Serena - vegetable rennet - Spain

5. natural microflora as starter culture,
   example: Mozzarella di Bufala - Italy

6. unique or traditional cheese making technology,
   example: Ragusano - Sicily, Italy

7. unique natural aging environment, and
   example: Formaggio di Fossa - Italy
   Roquefort - France

8. unique shapes or ingredients.
   examples: Le Pouligny Saint-Pierre - France
   Queso Tetilla - Spain
   Queso Cebreiro - Spain
   Queso San Simon - Spain

Any other factors that make a cheese variety unique to a defined geographic region can be considered in the application for the PDO designation. A cheese variety does **not** have to be unique in all the characteristics mentioned above to achieve PDO designation. Examples (i.e., pictures) of specific PDO cheese varieties that display one or more these characteristics will be presented.

Will Traditional European Cheese Varieties Survive?

YES! They will survive as long as the people and culture of various geographic regions within Europe survive. The PDO designation provides some protection for the name and defines the connection of unique cheese varieties to their original geographic region of production. This is one small step. Most of these cheeses will never compete with industrial cheeses based on price. These specialty cheeses will always be higher in price than mass produced industrial cheeses, but these special cheeses provide the consumer with added value in their unique sensory characteristics and their story of the culture of the people that produce them. It is the responsibility of the producers of these specialty cheeses (through their PDO consortium) to develop appropriate good manufacturing procedures and utilize the principles of HACCP (Hazard Analysis Critical Control Point) within the traditional cheese manufacturing procedures to produce cheeses that meet the consumer's expectations for product quality and safety.

Table 1. Number of PDO cheeses in various countries and PDO cheese production as a proportion of total cheese production within that country [1].

European Union States	Number of PDO Cheeses	Percentage of total cheese production in that country
France	33	10.4
Italy	30	49.1
Greece[2]	20	42.9
Spain	11	4.5
United Kingdom	10	NA[3]
Portugal	10	NA
Netherlands	2	7.6
Belgium	1	NA
Austria	1	NA
Germany	0	0
Denmark	0	0
Sweden	0	0
Luxembourg, Ireland, Finland	0	0
Total	118	

[1] Sources: a. del Latte 1996, INRA; Agric. Economics and Social Research Institute, Kifissa, Greece; Boerenkaas Assoc.
[2] PDO industrial cheeses only.
[3] Not available

Table 2. Top four PDO cheese for France, Italy, Spain, and Greece based on quantity of cheese produced[1].

European Union States	Top Four PDO Cheese Varieties
France	Cantal
	Comte
	Reblochon
	Roquefort
Italy	Gorgonzola
	Grana Padano
	Parmigiano Reggiano
	Pecorino Romano
Spain	Idiazaba
	Mahon
	Manchego
	Serena
Greece[2]	Feta
	Kasseri
	Graviera Agrafe
	Graviera Kreta

[1] Sources: a. del Latte 1996, INRA; Agric. Economics and Social Research Institute, Kifissa, Greece; Boerenkaas Assoc.

[2] PDO industrial cheeses only.

Specialty Cheese: What, Where & How?
Highlights of the open house

Chef Allen Hendricks
Culinary Consultant

Introduction

I. What makes a cheese a "Specialty Cheese"

A) Definition of Specialty Cheese

1) *Dan Strongin, Chef/Retailer/Consultant*
"Imported, imported style, and fine domestic varieties of quality cheese with unique flavor, texture, history, or origin, merchandised as a premium product."

2) Definition of Specialty Cheese *Steve McKeon, Owner/Manufacture*
"A cheese served at a special occasion or used in a special context"
"Individual Interpretation" or "Eye of the beholder" or "One Persons Treasure"

3) Definition of Specialty Cheese *Rob Gokey, Director of Marketing, Bongrain Cheese USA*
"The perception of the consumer as not an everyday item. It's a special treat for the family or some special entertaining event that they have coming up."

4) "Specialty Cheese is Served by Special People"
"Make a special cheese, that makes the customer feel special"

B) What factors make a cheese a "Specialty Cheese".

1) WSCI Definition of Specialty Cheese
"Specialty food products as used herein shall mean: foods, beverages or confections meant for human use that are of the highest grade, style and/or quality in their category"

2) Their specialty nature derives from a combination of some or all of the following qualities:

a) Their uniqueness
(String Cheese)
b) Exotic origin
(Parmesan)

c) Unusual application or use (Queso Blanco being fried)

d) Particular processing
(Swiss production in copper kettle)

e) Design
(Alouette Elegante)

f) Limited Supply: Production Amount of less than 40 million pounds per year. (WSCI)

g) Extraordinary packaging or channel distribution/sale

h) High quality

II. Production and Consumption

A) Of the 27.4 LB. per capita cheese consumption in 1995
11.8 LBS. American Type Cheese
10.3 LBS. Italian Types
5.3 LBS. Other
Source: Food Distribution Magazine, June 1997

B) Cheese consumption is up 1/2 of LB. per capita or about 27.73 or 1.7% growth from 1995 to 1997
Source: USDA

C) Cheese Production Increases:
June 1997 622 Million Pounds 4.5% increase over 1996
Source: National Agriculture Statistics Service (NASS)

D) It is projected by the year 2005, annual cheese consumption per capita will reach 35 pounds
Source: The Food and Agricultural Policy Research Institute (FAPRI)
1996 U.S. Agriculture Outlook,

E) Wisconsin Specialty Cheese production has increased by 40% since 1993

	Wisconsin Specialty Cheese Production
1995	98.9 million pounds
1996	116.1 million pounds

An increase of 17.2 million pounds, or a5.8% increase
Source: Wisconsin Agricultural Statistic Service (WASS)
Cheese Market News Vol. 17, No. 18 , June 13, 1997

F) 1995 43 Wisconsin plants manufacturing specialty cheese
1996 50 Wisconsin plants manufacturing specialty cheese

Source: Wisconsin Agricultural Statistic Service (WASS)
Cheese Market News Vol. 17, No. 18 , June 13, 1997

III. Where is specialty cheese being used?

A) Foodservice - Chefs

1) Foodservice defined - "prepared meal that is not traditional home cooking"

2) Almost 2/3 of all foodservice meals are consumed away from the place of preparation.

3) Foodservice cheese sales are predicted to increase from 30.3% in 1995 to 30.5% by 1998. Total sales reaching to $7.4 Billion dollars.
Source: Grocery Marketing; "Say Cheese, Think Flavor," June 1996

B) Restaurants:
1) White Table cloth
2) Chain Concept
3) Ethnic

C) Caterers / Institutional feeders:
1) Dorms
2) Colleges & Universities
3) Hospitals,
4) Government cafeterias
5) School foodservice.

D) Cruse ships:
1) Use specialty cheese often
2) Captive audience that are served many meals
3) Buffets

E) Ethnic foods drive specialty sales
1) Chain concept Restaurants
a) Mexican, Tex-Mex, Southwestern
b) Mediterranean
1) Pasta Dishes using grating cheeses
2) Salads - Greek using Feta

c) Pizza's - Using non-traditional cheeses or cheese blends
Such as Blue Cheeses

2) Independent Restaurants
3) Retail markets & delis

G) Traditional cheese dishes like
1) fondue
2) Rarebit

H) Classic sauces:
1) Mornay, used as the bases for
a) Soufflé

2) Cheese sauce used
a) Served over vegetables
b) Mac an cheese
c) Cheese Soup

IV) Where are they using it? What are they doing with it?

A) In general, traditional menu items using non-tradition ingredients

B) Trading out the tradition cheese for non-traditional cheese or adding menu items with specialty cheeses

C) Breakfast items
1) Omelets

D) Sandwiches
E) Salads
F) Entrees
G) Appetizers
H) Desserts
I) Cheese coarse or Cheese Plate
J) Party trays
K) Box lunches
L) Buffets and Receptions

V. Restaurant Chefs Drive the Retail Market
They Create the menu items. Restaurant customers shop retail outlets for the ingredients to recreate the menu item at home, thus driving the retail market.

VI. Cheese Sales

A) Retail Cheeses sales reached 8.5 Billion in 1995.
It is expected to reach 9.4 billion by 1999.
Source: Grocery Marketing; "Say Cheese, Think Flavor," June 1996

B) At one time specialty cheese were sold primarily in "Specialty Shops" But supermarkets are continuously adding additional cheeses

1) Generally offer service cheese counters
2) Tend to stock more specialty varieties then a supermarkets

C) In supermarkets with over $2 Million in annual sales:
Specialty cheese sales have increased 8% from 1995 to 1996.
In the same period, the total cheese category increased only 1.5%.
Source: Dairy Field; "Special Niche for Cheese Producers," March 1997

D) In supermarkets with over $2 Million in annual sales:

Cheese:	Pounds:	Growth %:
Neufchatel	25.9 million pounds	4.8%
Feta	5.9 million pounds	13.2%
Blue Cheese	2.7 million pounds	4.2%
Camembert & Brie	2.4 million pounds	7.1%
Edam	632,400 pounds	29.1%
Gorgonzola	147,300 pounds	29.5%

Source: A.C. Nielsen data for the 52 week period ending June 14, 1997
4.2% increase over the same period last year

E) 31.2% of supermarkets had cheese shops or cheese centers in 1996.
vs. 1995, 26.9%
Source: Progressive Grocer's Annual Report of the Grocery Industry

F) Nacho cheeses grew 1,585%
1)Cross-branding has helped boost these sales.
1995 Kraft introduced Velveeta line w/ Pace salsa
Source: Dairy Field, "Consumers, Processors Team Up for Continued Cheese Category Growth," December, 1996

G) According to Sargento, stick and string cheese sales are up 10% from 1 year ago.
1) Families with children are almost 4 times more likely to buy string cheese than those without.
Source: Cheese Market News, "MooTown Snacks introduces Twirls," March 14, 1997

H) Goat cheese sales in supermarkets increased 29.4% in 1995 from the same period in 1994.
Source: National Cheese Institute Cheese Market Research Project with A.C. Nielsen

VII. Full Service vs. self-service Cheese

A) 1989 Full service supermarket cheese counters sold an average of 528 LBS. per week. The latest survey shows a 50% drop to about 255 LBS. per week, due to the trend towards self service cheese departments

B) The 1995 survey compared manned cheese centers to self service centers. There seems to be a direct correlation in sales: 50% fewer man-hours and about 50% fewer sales

Marketing Point: Focus on Retailers that have service cheese counters
Source: Cryovac survey on trends in specialty cheese,
The Gourmet Retailer, October 1995.

C) Specialty cheeses are becoming more abundant in the slicing section of deli cases. Cheeses like Havarti, Aged or Sharp Cheddar, Brick, Smoked Cheeses, Gruyere, Gouda and Edam.
Source: Deli Business, "Slicing Up Bigger Cheese Profits In the Deli." Winter '97

VIII. Where to look for the trends

A) Publications
1) Restaurant Trends - Foodservice Trade Publications
2) Retail Trends - Retail Trade Publications
3) Consumer trends - Consumer Magazines

B) Trade & Marketing Associations
1) WMMB
2) IDDA

C) Do your own market research
1) When dinning out look at the menus - See what is being offered
2) When traveling to different cities;
a) visit supermarkets and specialty stores

IX. The "Open House" reception

A) Educational
B) Enjoyable evening event

C) Selection of the cheeses
1) Pick award winning cheeses
a) WCMA World & US Championship Cheese contests
b) ACS Annual Judging

2) Solicit donations from RP Customers

3) WMMB supports the event by sponsoring Wisconsin Specialty

Cheeses.

D) Beverage parings
   1) Wine
   2) Beer, ale, etc.

E) Signage:
   1) Foodservice - used on buffets and food bars
      a) Cheese name
      b) Flavor descriptions

   2) Retail - used to promote cheese sales
      a) Cheese name
      b) Price
      c) Flavor descriptions
      d) Suggested uses

Unique Cheeses and Some Unique Ideas

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Topics

An overview of some unique cheeses I have seen
Some specific unique cheeses
- Tvaruzky
- Gryficki and Rolada
- A Post Ripened Milled Cheese
- A Naturally Ripened, Process Cheese with a filling (Jim's Dream Cheese)

Introduction

Over the last six years it has been a real pleasure to work in the Specialty Cheese Program, at the Center for Dairy Research. A program which has been funded entirely by the Wisconsin Milk Marketing Board. During that time I have had the opportunity to discover some very unique cheeses. I would like to share a few of these with you.

An Overview

We don't tend to think of process cheese as unique, may be we even think of it as boring, but these Magic Circus animal faces from France would catch the eye of any young child. Cheese in a tooth-paste tube, it's really not all that unique in Europe and not a bad idea. How about putting a layer of ash in the middle of your cheese! This popular cheese is from France and is called Mobier. Next is a layered cheese called Royal Windsor from the people at Long Clawson in England. It is a combination of layers of Stilton and "Cheddary Cheese, flavored with Elderberry wine." From Wisconsin both a great idea and a great football team - Green and Gold Curds.

How about truly environmental packaging Banon cheese from France, wrapped in leaves. Salami anybody! Another great way to package from Poland. Put a little swirl in your life with this cheese from the people at Long Clawson. This design cheese from Switzerland won a prize at the Natwitch International Cheese Show in England. Finally a cheese from Italy that is my favorite. Smoked Provolone from Italy, shaped into pigs.

Czech Cheeses - Tvarusky (made from Tvaroh)

First I would like to say that the information which I received regarding this cheese came from Ed Schuch from Green Bay, WI. Ed had the opportunity to travel to the Czech Republic and visit the state dairy school, where they train cheese makers.

In the slides you are seeing the students at the school manufacture "Old Czech Cream

Cheese", another unique Czech cheese. But now to Tvarusky!

In the Appendix you will find procedures for Tvaroh (the precheese) and Tvarusky.

The manufacture of Tvaroh is very similar to Bakers Cheese and is not unique in itself. It is when it becomes Tvarusky that it becomes unique.

The steps are as follows:

1. Grind the Tvaroh (cheese of different ages may be used)
2. Fill into a large bin and walk on the cheese with large pads on feet to compact it (bins hold about 20 ton)
3. Store in bin for about 3 months
4. Press into small round doughnut shapes
5. Ripen on shelf
6. Eat – normally fried or dipped in beer

Polish Cheese – Rolada

The information for this cheese comes from the Polish Artisan Cheese seminar which we held at CDR. We normally try to hold two of these seminars per year. One will be focused on a specific country and it's cheeses and the other on a facet of cheese making .

Once again the procedures for these cheeses are found in the appendix of this presentation.

The steps are as follows:

1. A pre-cheese is manufactured (Gryficki)
2. Fresh cheese and ripened cheeses are blended
3. Cheeses are placed into a hot bath to cook and mold (no salts are added)
4. Cheeses are smoked

As you can see from the last two cheeses the idea of blending ripened cheeses and repressing is not a new idea in Europe. This concept leads me to some cheese's we have explored at CDR.

Post Ripened Milled Cheese

A post ripened milled cheese is a cheese which has had the whey drained off and is pressed into a preliminary shape. It is ripened to a desired flavor. At this point it is remilled, ground or shredded and pressed into it's final shape.

Some examples of this type of cheese are:

1. Polish (Bunz to Brynza)
   Brynza (cow and sheep milk mixed)
2. England (Patrick Rance - Long Clawson)
   Ingredients added
3. CDR (Blue Jack)
   Blue mold added
4. CDR (Low fat)
   Normal fat (high flavor) and low fat blended

In conventional cheese manufacture the following steps are used:

1. Manufacture the cheese (add herbs, spices, molds, other ingredients)
2. Ripen the Product
3. Sell the product

In the post ripened method the following steps are used:

1. Manufacture the cheese
2. Ripen the product
3. Mill, grind, or shred the cheese
4. Add ingredients
5. Repress into final shape
6. Sell the product

The advantages of this process is as follows:

1. No risk in cheese with ingredients during ripening process
2. Can control flavor of finished product
3. Can control cheese inventory (made on demand)
4. Can add mold at a remote location

The disadvantages of this process are:
1. The question of legality.
2. Risk of contamination during post ripened milling

And now on to my dream cheese.

A Naturally Ripened White Mold Process Cheese (with a filling)
Jim's Dream Cheese

Almost twenty years ago, I developed a taste for white mold cheeses like brie and camembert. Like many other lovers of that type of cheese I soon discovered that these cheeses go very well with fruits. At a meeting I attended, I happened to mix some lemon preserve with the brie which I was eating and instantly fell in love with the taste. Some of you might have also tried similar things, like taking a piece of brie, microwave it for about 15 seconds and then pour maple syrup over it. Well being a cheese maker, I started my quest for the ultimate cheese, a white mold cheese with a filling.

Due to fact that fruit contains high levels of natural sugars, it is very difficult to add fruit to cheese products. This is because of the fermentation these sugars might cause. The answer came when on a trip to Europe when I was introduced to a white mold product which was made from process cheese. I was told that it was made by a company in Spain and was also patented in Europe by that company. I was also told that it was not widely accepted. This did not surprise me, given the tradition and availability of good natural white mold cheeses in Europe. It did however answer the question for me as to how to make my dream cheese.

The following steps would be needed:

1. Blend cheeses to desired flavor
2. Add salts and water
3. Cook
4. Pour first layer
5. Cool till solid
6. Add layer of filling (smaller then first layer)
7. Pour remaining cheese
8. Cool till solid
9. Inoculate with mold
10. Incubate
11. Wrap and sell

Many problems have the potential to be solved:

1. Since the product is cooked, there should be no late fermentation
2. Cheese could be blended to produce correct flavor, moisture and pH
3. Shelf life should be extended
4. Should eliminate unripened center

We are currently working on this cheese at CDR.

Thank you for your time and I hope you have enjoyed this unique look at cheese. If you have an interest in any of these cheeses you may contact me at the CDR (608) 262-2253 .

Manufacture of Tvaroh

1. Pasteurized skim milk
2. Set at 31 degrees C (88 degrees F) with lactic culture.
3. 14-16 hours
4. Whey acid= 28 SH (.63% acid)
   Mass acid =38 SH (.86% acid)
5. Into bags
6. Drain
7. Final product= 25% T.S.
8. PH 4.2 ?
   SH 90-100 (2.0-2.2 % acid)?

(Resembles Baker's cheese)
Tvaruzky basic ingredients:

TVAROH of 3 different ages: fresh; 3-4 weeks old; 3 months old.
30-34% dry matter in Tvaroh.
Acidity up to 160 SH (3.6%)

First step - grind Tvaroh in grinder. Add 4% salt. Goes into large bin "walked on in bin", during grinding process. Done to pack ground material in bin, to prevent air pockets in ground material which might allow growth of aerobic bacteria. Each bin may contain 20 tons of material. After bin is full and packed, heavy weights are placed on top of material in the bin. Then held up to 3 months to age. (Some packaged at 3 weeks) When aging process is done, material is pressed into small flat, round pieces, about 2 inch thick and 1-3/4 inch in diameter. Pressing done by machine. (In earlier times, pressing was done by hand, using a hand press. One person would press 300-500 Kg per day. (660-1100 pounds)

The 3 ages of Tvaroh are mixed in about equal proportions for pressing, but proportions are changed during the year according to seasonal composition of the Tvaroh used (calcium, sodium composition)

Sodium bicarbonate is added to material to lower acidity from 160 SH (3.6% acid) to 120 SH (2.7% acid), to allow surface ripening to begin. B. Linens and yeast, the surface ripening agent, would grow in high acid, 160 SH, environment. After pressing into forms, pieces are placed on boards, and the boards, with pieces are placed in racks, to dry for 3-4 days at less than 31 degrees (88 degrees F). Culture is on boards and in this environment it begins to grow on the pieces.

After drying/culture growth periods of 3-5 days, pieces go to washer to be washed with water or whey. (Some people buy the product, dip in beer, age for few days in refrigerator for different effect.)

Packaged when 2/3 cured. Finish cure in package. Shelf life= 2-4 weeks, depending on temperature of storage. Usually eaten with bread and butter, as spread or coated and fried, with eggs, etc. Also onion.

Gryficki cheese

Manufacturing Gryficki was started in 1973 in Gryfice dairy (province of Szczecin). The technology was developed and implemented by the Institute of Dairy Technology (Warsaw).

The shape of Gryficki resembles smoked ham. Its rind is light-brown and the weight is 0.3 - 1.0 kg. The cheese blocks are wrapped in a net. The cheese body is homogenous or slightly cracked and fibrous, elastic or slightly crumbly. Single eyes are acceptable. The flavor is slightly sour, slightly piquant, with a distinct smoked aroma. It is manufactured as a full fat cheese (45% fat in DS, 45% moisture).

Add $CaCl_2$ and starter (1.5 - 2.0 %) to pasteurized and standardized milk. The milk is warmed to 34∞C and rennetted to produce a medium-firm curd during 25 - 30 min. The curd is cut into 6 mm cubes. The curd-whey mixture is scalded at 38∞C. The rate of the temperature increase should not exceed 1∞C/2 - 3 min. Draining the curd takes 20 min. Then the stirrers are stopped and pressing is started. The settled grains are consolidated on the bottom of the vat into a block of 25 cm high. The whey is partly taken-off, the block is covered with cheesecloth, sheets are placed on the block surface and a load of 5 kg/kg cheese is applied during 25 - 30 min. The moisture content in the cheese after pressing should be 55%. The cheese is maturated (acidified) for 4 - 5 h at 25 - 30∞C to obtain an acidity of 70 - 75∞SH. This step can be carried out directly in the vat or the cheese block may be cut into blocks of 50x50 cm which are subsequently placed in a room at 25 - 30∞C. In the case of leaving the cheese overnight, it should be cooled down to 2 - 5∞C to interrupt the fermentation process.

The acidified cheese mass is cut in a special cutter to 1 -2 mm slices. A portion of the slices (4 - 5 kg) is placed in a wicker basket and immersed for 30 - 40 s in a bath at 70 - 72∞C during a constant stirring. The high cooked cheese mass acquires a pasty texture. The bath is composed of 6 kg salt, 20 l whey and 80 l water; the acidity of 14∞SH. The cooked cheese is cut into portions of 1 kg and quickly shaped manually to obtain the shape of ham baton. Afterwards the cheese batons are immersed in brine (16 % salt, pH of 5.4) at 20 - 25∞C for 1 - 2 h. The salted cheeses are tied up and hanged on special stands at 10 - 15∞C for 24 h.

The next day the cheeses are smoked in a smoke made from burning hard wood chips. The temperature should not exceed of 40∞C; the best results of smoking are obtained at 20∞C. Smoking takes 2 - 4 days. During this step a rind is formed which protects the cheese against mold growth as well as moisture loss.

The smoked cheese batons are immersed in a yellow paraffin bath at 130∞C and then hanged on special stands in the ripening room at 10∞C for 5 days. The ripe cheeses are labeled, wrapped in paper and placed in cardboard boxes.

Gryficki has a long shelf life as well as favorable flavor and appearance features. That is why it is manufactured in many Polish dairies.

RRolada ustrzycka cheese

Manufacturing Rolada ustrzycka (spelling: *r-o-l-a-d-a oo-s-t-sh-y-tz-k-a*) was started in 1976 inn Ustrzyki Dolne dairy (province of Krosno). Rolada ustrzycka is a special cheese manufactured frrom ready cheese (not from cheese milk). The cheese shape is similar to the baton of ham. The wweight of the cheese baton is 0.3 - 1.0 kg. The rind is light-brown, paraffined or wrapped in foil. On thhe cross-section there are visible irregular stripes which resemble a collar (rolada means collar, rcolled meat). The texture is soft, slightly loose, elastic or slightly crumbly. The flavor is aromatic, aggreeable, slightly salty, nutlike, slightly sour, slightly piquant, slightly smoked and garlicky. Rolada uustrzycka is manufactured as a fat cheese (40% fat in DS, 50% moisture). *The raw material used is anny ripe rennet cheese of good quality blended with fresh trimmings (parings) of any ripened rennet cheese obtained directly after manufacturing.*

A ripe cheese is cleaned, wrapping material (PVA), the rind and the mold infected holes are reemoved. Then the cheese is cut into smaller portions and subsequently into thin slices (1 - 2 mm). TThe batch is composed of 70% sliced ripe cheese and 30% fresh cheese trimmings or cut fresh cheese (e.g. manufactured according to the procedure for Gryficki). The ingredients are blended ccarefully and high-cooked. The cooking bath contains 3 kg salt, 20 kg deproteinized whey (50∞SH) and pasteurized water up to 100 kg. Alternatively, an aromatic bath containing additionally 0.4 kg dried and cut garlic is also used. A portion of the cheese blend (3 kg) is placed in a perforated container and immersed in a hot bath (65 - 70∞C) for 45 s. The cheese blend is stirred during cooking. When the mass is plastic, the container is taken out of the bath and emptied, the mass is kneaded on thhe cheese table and shaped manually into cylinders. The hot cooking and shaping (molding) can allso be carried out in a special flow device.

Afterwards the cheese batons are immersed in brine for 1.5 h (16 - 18% salt,), tied up and haanged on the sausage trolleys for dripping and consolidation (12 h, 14∞C). Then cheese is smoked im a natural smoke at 25∞C to produce a light-brown rind. The smoked batons are paraffined at 80∞C or coated with plastic or wrapped in aluminum foil, weighed, labeled, placed in plastic containers lined with parchment paper and transferred to storage at 10∞C for 3 days.

The use of lysozyme:

A natural and efficient solution to prevent the butyric late blowing in cheese

Presentation given to the
Marschall Italian & Specialty Cheese Seminar
Gilles LAGARDE
September 18, 1997

The lysozyme application in cheese

- The butyric fermentation
- The lysozyme activity against the agent of the butyric fermentation: Cl. tyrobutyricum
- The lysozyme action and efficiency in cheese
- The practical use of lysozyme
- The legal status of the use of lysozyme
- Conclusion

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The butyric fermentation

- origin
- consequences on the cheese quality
- preventive and curative treatments: advantages/disadvantages of each solution

The butyric fermentation

- **origin**
- consequences on the cheese quality
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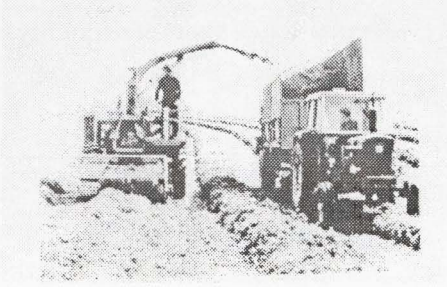
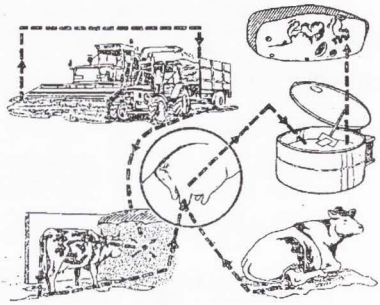
The silage: main origin of the butyric contamination of milk

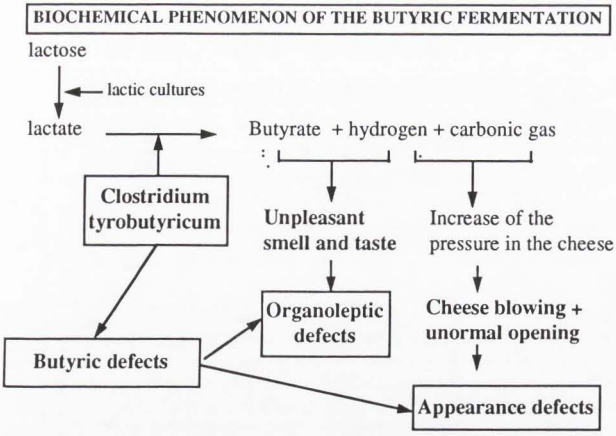


The butyric cycle: from silage to cheese

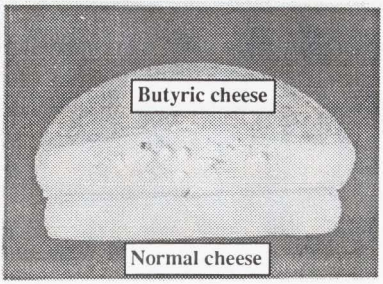


The butyric fermentation

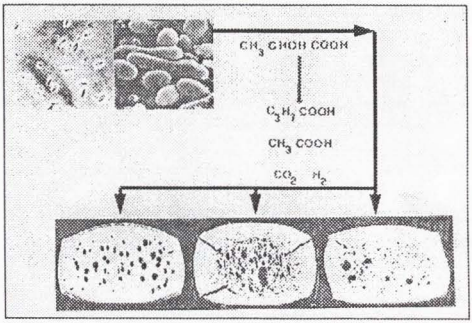
- origin
- **consequences on the cheese quality**
- preventive and curative treatments: advantages/disadvantages of each solution



Consequences of the butyric fermentation on the cheese quality



Example of a butyric late blowing in a Grana cheese



Relative importance of the butyric contamination / total milk flora

- total milk flora :
  10^4 to 10^5 c.f.u. / ml of milk
- butyric contamination :
  10^2 to 10^4 spores / liter of milk or
  0.1 to 10 spores / liter of milk
- **the butyric contamination is 10^4 times less important than the total milk flora**

The butyric fermentation

- origin
- consequences on cheese quality
- **preventive and curative treatments: advantages/disadvantages of each solution**

Solutions available to cope with the butyric fermentation

- **Preventive:**
  - **actions at the farm level to improve the milk bacteriological quality**
  - –
  - **selective milk collect**
- **Curative:**
  - **adjustment of technological parameters: NaCl, pH**
  - **physical processes: creaming, bactofugation**
  - **chemical processes: NO_3, Nisin, H_2O_2**

The lysozyme application in cheese

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- **The lysozyme activity against the agent of the butyric fermentation: Clostridium tyrobutyricum**
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The lysozyme activity against Clostridium tyrobutyricum

- Clostridium tyrobutyricum
- lysozyme enzymatic activity
- lysozyme action on Cl. tyrobutyricum

The lysozyme activity against Clostridium tyrobutyricum

- **Clostridium tyrobutyricum**
- lysozyme enzymatic activity
- lysozyme action on Cl. tyrobutyricum

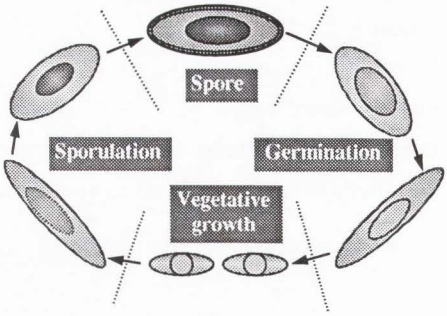
Clostridium tyrobutyricum : main features

- Gram + bacteria
- bacillus shape under a vegetative form
- strictly anaerobic
- uses a limited number of sugars
- can metabolise the lactates as source of C

Clostridium tyrobutyricum : conditions of growth

- Optimum temperature : 35° C
- Optimum pH : 5.8
- Cl. tyrobutyricum sporulates if medium conditions are unfavorable
- Cl. tyrobutyricum has a development cycle

Development cycle of Clostridium tyrobutyricum



Clostridium tyrobutyricum : enumeration methodology

- Principle :
  test of Briant-Burkey which uses the **main metabolic features** of Cl. tyrobutyricum
  - **Thermoresistance**
  - **fermentation of lactates as source of Carbon**
  - **production of gas**
  - **development under strict anaerobic conditions**

Clostridium tyrobutyricum : enumeration problems

- **Problems :**
  - **the method is time consuming**
  - **the incubation is long:7 days**
  - **the method is not specific enough**
  - **the enumeration is not accurate (MPN tables)**
- **Consequences :**
  - **no efficient tool to select the milk**
  - **impossible to adapt a curative treatment to the level of contamination**

–

The lysozyme activity against Clostridium tyrobutyricum

- Clostridium tyrobutyricum
- **lysozyme enzymatic activity**
- lysozyme action on Cl. tyrobutyricum

Origin and presence of lysozyme

- Discovered by Fleming in 1922
- widely present in nature
- not present in cow's milk
- can be found in humans in large amounts in tears, saliva and mother's milk
- used in different drugs
- the most important natural source is the egg white

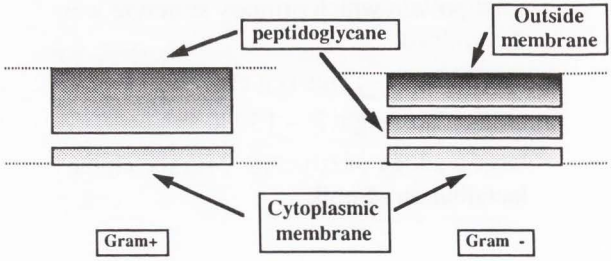
The lysozyme molecule

- First protein which primary structure was determined
- egg white lysozyme is a chain of 129 a. a.
- molecular weight is ~ 15000
- large analogy of structure with the alpha-lactalbumin of milk

Lysozyme enzymatic activity

- The substrate is a component of the bacteria cell wall: the peptidoglycane
- the peptidoglycane is more accessible in the cell wall of gram+ bacteria
- lysozyme activity is measured on a very sensitive bacteria (Micrococcus lysodeikticus)

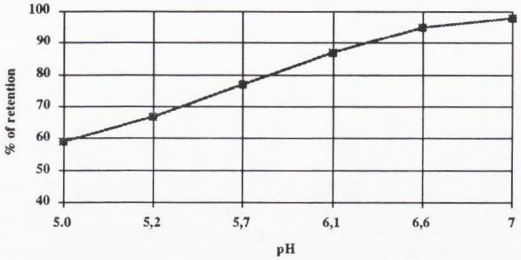
Structure of the cell wall of gram+ and gram- bacteria



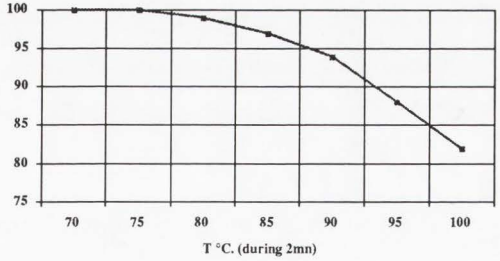
Lysozyme sensitivity to physical factors

- Isoelectric point is between 10 and 10.5
- resists to high temperatures (up to 80 °C) at acid pH
- optimum activity at pH ~ 6
- optimum activity at 55-60 °C

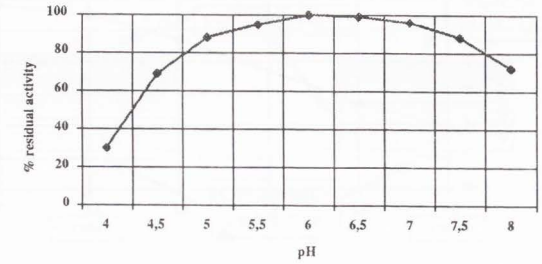
Influence of the pH on the lysozyme retention in milk



Influence of the temperature on the lysozyme activity



Influence of the pH on the lysozyme activity



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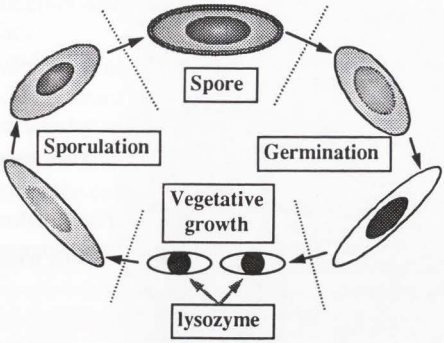
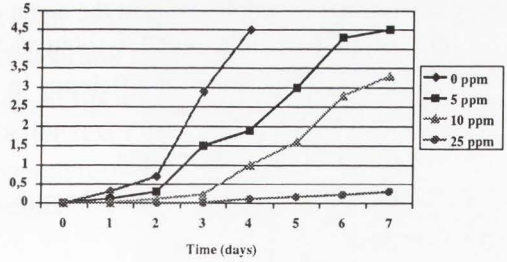
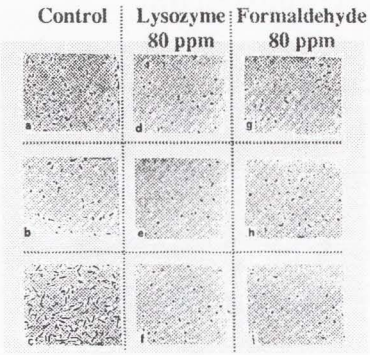
Lysozyme action on Clostridium tyrobutyricum



Inhibition power of lysozyme on the growth of Cl. tyrobutyricum



Influence of lysozyme on the germination of spores of Cl. tyrobutyricum



The lysozyme application in cheese

- The butyric fermentation
- The lysozyme activity against the agent of the butyric fermentation: Cl. tyrobutyricum
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How does lysozyme work in cheese?

- Lysozyme is retained in the curd by electrostatic attraction with the casein micelles (< 5 % lost in whey)
- lysozyme is not active on the spores of Cl. tyrobutyricum as long as they do not germinate (no activity in milk)
- lysozyme remains active in the curd and can disrupt the cell walls of the vegetative forms of Cl. tyrobutyricum if the medium conditions become favorable to the germination of the spores

Action and efficiency of lysozyme in cheese

- Greatly reduced production of butyric acid: **no off flavors**
- greatly reduced production of gas: **less cracks and less opening**
- longer ripening time: **better flavor and better texture**
- overall better grading of the cheese: **higher value**

A successful track record of 15 years of use in Europe

In different countries:

- FRANCE
- ITALY
- GERMANY
- SPAIN
- PORTUGAL
- DENMARK
- HOLLAND

In different types of cheeses:

- **hard cheeses:**
  - grana padano
  - emmental/swiss
- **semi-hard cheeses:**
  - gouda
  - mimolette
  - Manchego
- **soft cheeses:** Brie
- **processed cheeses**

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The practical use of lysozyme

- Diagnosis of the origin of late blowing defects
- determination of the optimal dosage
- important recommendations

Possible origins of blowing problems in cheese

	Source of contamination	Fermentation by-products	Consequences on the cheese quality
Hetero-Fermentative Bacteria	Milk Equipment Water Cultures	CO_2 Acetic acid	Early opening / Excessive opening
Coliforms	Milk Equipment Water	CO_2	Early opening / Blowing during the pressing
Propionic Bacteria	Milk	CO_2 Propionic acid	Late opening/blowing
Butyric Bacteria	Milk	$H_2 + CO_2$ Butyric acid	Late opening/blowing

How to determine the optimal lysozyme dosage ?

- Enumeration of Cl. tyrobutyricum is too slow (7 days) and inaccurate (sigma = 1log)
- it cannot be used to adjust the dose of lysozyme a priori to the level of contamination
- the recommended dosage of **25 ppm** (or **0.2 Lb. / 1000 gallons** of milk) is a "safety dosage"

Adjustment the of safety dosage

- according to the season: milk is more contaminated in winter time
- further to other milk treatments: use of other inhibitors or physical elimination of spores
- to comply with economic constraints: **the cost of treatment @ 25 ppm is 1,5 cent/lb. of cheese.**

Important recommendations for a successful implementation

- **Confirm the butyric origin of the late blowing (VFA analysis)**
- **choose the optimal lysozyme dosage**
- **check the starters sensitivity at this dosage**
- **prepare the lysozyme solution carefully at the proper pH (above 4.6)**
- **add the lysozyme solution to the milk at the right stage:**
  - **after all the heat treatments**
  - **as early as possible before the addition of the rennet and of the cultures**

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The legal status of lysozyme

- Approved as a preservative (E 1105) in the new E.U. Directive on food additives
- G.R.A.S. affirmation petition accepted for filing in 1989 and still pending in the U.S.
- Petition for the use in cheese recently filed in Canada

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The key advantages of using lysozyme to prevent the butyric late blowing in cheese

- **Natural** and **safe** product
- **very selective** spectrum of action: usable in all types of cheeses
- **easy to implement** without specific equipment: usable by all dairies of any size
- **flexible**: can be used in combination with other curative treatments (bactofugation)
- **very efficient** against the butyric blowing
- **successful** track record of use during 15 years in Europe in different types of cheese

Manufacturing Lower Fat and Skim Milk Mozzarella Cheese

Carol M. Chen
Cheese Ingredient Applications Coordinator
Wisconsin Center for Dairy Research

Introduction

Consumers knowledge of the link between fat intake and health have heightened their receptiveness to the concept of reduced fat foods, which in turn has lead to the development of a variety of lower fat cheeses. A typical American diet derives the following percentage of calories from carbohydrates (45%), protein (15-20%), and fat (35-40%). The American Heart Association recommends that caloric intake be 55% from carbohydrates, 15% from protein and 30% or less be from fat. Taking a look at low moisture, part-skim (LMPS) Mozzarella cheese, one serving (30 g) contains 5 grams of fat and 8 grams of protein, this correlates to 58% of the calories from the fat portion. Consumers concerned about their fat intake are looking for lower fat cheese alternatives. Now, you, the cheese manufacturer have the challenge of producing lower fat Mozzarella cheeses that live up to the consumers expectations.

This talk today will review three topics in the development of reduced fat and skim milk Mozzarella. Before developing a manufacturing protocol, it is important that the nutrient claims are understood, so a target cheese composition can be determined. Secondly, the cheesemaker needs to consider the cheese end use. Since most Mozzarella cheese is used as an ingredient on pizza pies, it is critical that not only the flavor, but the physical properties be desirable. This is one of the greatest challenges in the manufacture of lower fat Mozzarella cheeses. Lastly, small scale manufacturing protocols for reduced fat and skim milk Mozzarella will be discussed.

Nutrient Claims

Maximum fat contents in nutrient claims are determined by either a percentage reduction from the reference food (reduced fat, light) or a maximum amount of fat per serving (lowfat, nonfat). A nutrient claim of **reduced fat** indicates that the cheese contains at least 25% less fat than the reference cheese. To make a **light** claim, the fat content must be reduced by at least 50% because cheese derives 50% or more of its calories from fat. **Lowfat** cheese contains 3 grams or less of fat per 50 grams or 6% fat on a wet basis. **Nonfat** cheese contains 0.5 g or less of fat per 50 grams or 1% fat on a wet basis. The milk used to manufacture nonfat cheese typically contains 0.08% milkfat or less. However in some manufacturing facilities, the separated skim milk has a slightly higher fat content (range 0.09 - 0.12% milkfat). If this milk were used to manufacture cheese the resulting cheese would have 1.5 - 2.0% milkfat, which then could not be legally called nonfat cheese. These may be called skim milk cheese.

Determining the maximum fat contents for Mozzarella cheese nutrient claims is complicated by the four U.S. Standards of Identity of Mozzarella: Mozzarella, low moisture Mozzarella, part-skim

Mozzarella and low moisture, part-skim Mozzarella (See Table 1). The maximum percentage of fat in reduced fat Mozzarella ranges from 12.0 - 13.5% depending on the moisture content of the cheese. For example cheese composition of 13.5% moisture and 56% moisture, has a FDM of 30.7%. This cheese composition falls within the standard of part-skim Mozzarella and therefore must be called part-skim Mozzarella.

In October 1996, IDF submitted a Draft Standard for Mozzarella which defines two types of Mozzarella, low and high moisture. This will change and simplify the calculations for maximum fat content in nutrient claims.

Table 1. The complicated world of Mozzarella Cheese

	% Moisture	% FDM	25% Fat Reduction	50% Fat Reduction
Mozzarella	52-60	Min 45	13.5	9.0
Low Moisture Mozzarella	45-52	Min 45		
Part-skim Mozzarella	52-60	30-45		
Low Moisture Part-skim Mozzarella	45-52	30-45		
	Reduced fat	Light	Lowfat	Nonfat
Maximum fat to make nutrient claim	12-13.5% *	9%	6%	1%

Note
The maximum fat content is 13.5% if the cheese moisture is less than 55%, because the FDM is less than 30%. If moisture content is greater than 55%, then the FDM is greater than 30% and the cheese must be called part-skim Mozzarella. When the fat content is less than 12%, a cheese may be called reduced fat, regardless of moisture content.

Lower fat Mozzarella cheeses.

Mozzarella cheese's clean mild flavor and favorable physical characteristics make it well suited for use as an ingredient, especially on pizza pies. Manufacturers have the challenge of maintaining the desirable melt, stretch and chewiness characteristics without the development of excessive browning, blistering and skinning of LMPS Mozzarella in a lower fat Mozzarella product.

The physical characteristics of Mozzarella are largely dependent on the protein integrity with respect to cheese composition, cheese pH (demineralization) and degree of casein hydrolysis. Cheese is a continuous para-casein matrix with entrapped moisture and fat. The density of this protein matrix (spacial arrangement) and its chemical properties determines whether the body of the cheese is soft or firm. In addition, cheese pH (demineralization) and degree of casein hydrolysis are especially important in the overall melt and stretch characteristics of the cheese.

Physical Characteristic	Chemical or Compositional Factor	Correlation
Melt	% FDM	positive
	Cheese pH (demineralization)	negative
Stretch	Casein hydrolysis	negative
	Cheese pH (demineralization)	ideal ~ 5.25

Maintaining physical characteristics of LMPS Mozzarella in reduced fat and skim milk Mozzarella is dependent on increasing cheese moisture as well as optimizing other key chemical factors such as cheese pH and degree of casein hydrolysis.

An appropriate moisture range needs to be specified for cheeses at any given percentage of FDM. When the cheese moisture content is too low, the resulting cheese is dry and firm. If the moisture content is too high, the cheese will be soft and weak bodied. These cheeses are difficult to shred, may be too fluid when melted on a pizza pie and have a very short shelf-life. Table 2 gives target cheese compositions used at the Wisconsin Center for Dairy Research for the manufacture of LMPS, reduced fat and skim milk Mozzarella cheese.

Table 2. Target cheese composition of LMPS, reduced fat and skim milk Mozzarella cheese.

	Moisture	Protein	Fat	Salt	Component Total
Low moisture, part-skim Mozzarella	48.0 %	26.0 %	21.5 %	1.5 %	97.0 %
Reduced-fat Mozzarella	53.0 %	30.0 %	12.0%	1.5 %	96.5 %
Skim milk Mozzarella	62.5 %	29.5 %	1.5 %`	1.5 %	95.5 %

Cheese Fat Determination - Mojonnier vs Babcock

The AOAC specifies the use of a modified Mojonnier method (ether extraction) for determination of cheese fat content. However, due to limited resources and the time intensive nature of Mojonniers, most cheese manufacturers use a modified Babcock method to determine cheese fat content. In the Babcock analysis, concentrated sulfuric acid is added to digest proteins, this releases fat trapped within its matrix. The digested cheese is then centrifuged, tempered and the fat volume is read from the neck of a Paley bottle. It is well documented that the percentage of fat by the Babcock assay reads high for full fat cheese. In addition, our laboratories have shown that Babcock assay reads low on reduced fat and skim milk cheeses. We speculate that this is due to the smaller fat globule size and the higher protein contents in the cheese.

Table 3. Percentage of fat in cheese as determined by Mojonnier (ether extraction) and Babcock

Type of Cheddar cheese	% Fat		
	Mojonnier	Babcock	Difference
Full Fat	29.66	29.9	+0.24
25% RF	26.51	26.2	- 0.31
33% RF	20.70	20.2	- 0.50
50% RF	14.02	13.5	-0.52
75% RF	8.72	7.3	-1.42
Skim milk	1.42	0.6	-0.82

Manufacturing Reduced fat and Skim milk Mozzarella cheese

As you've already gathered producing a reduced fat or skim milk Mozzarella cheese is more than using part-skim or skim milk in a low moisture, part-skim manufacturing protocol. The key factors in the manufacture of lower fat Mozzarella cheese with desirable flavor and physical characteristics is increasing cheese moisture content, while maintaining a final cheese pH of 5.25 and limiting the degree of casein hydrolysis. How can this be achieved?

Table 4 describes key manufacturing steps of reduced fat Mozzarella used in small scale production. The high moisture content is achieved by a short manufacturing time, 70 minutes from renneting to milling (preacidification, fast rate of acid production) and a rigid curd structure (low pH at addition of milk coagulant, firm milk coagulum at cutting). Favorable melt characteristics can be attributed to demineralization (low curd pH at addition of milk coagulant) and a final cheese pH of 5.15 - 5.20. High mixer temperatures limit proteolysis, so cheese stretch qualities are maintained and shelf-life is extended.

Table 4. Key manufacturing steps for the production of reduced fat Mozzarella cheese.

Manufacturing Step	How	Why
Preacidification	Add acetic acid to cold raw milk to milk pH = 6.25	* shortens total cheese manufacturing time * lowers pH at addition of milk coagulant, thus more demineralization from casein micelle
Addition of starter culture	Add high level of starter culture (1.5% wt/wt) and high ripening temperature = 102°F	* shortens total cheese manufacturing time * lowers pH at addition of milk coagulant, thus more demineralization from casein micelle
Firm milk coagulum at cutting	pH at renneting = 6.05 Reduce milk coagulant level ~⅓ less (10 ml 2x coagulant /1000 lb milk) Setting time = 15 minutes	* lower pH = increased milk coagulant activity * reduce milk coagulant level so milk does not set too fast * firm milk coagulum at cutting help maintain high moisture content in cheese
Cooking Temperature	After cutting, allow 20 minutes to reach cooking temperature of 106°F	* cooking temperature only 4°F different than ripening temperature - helps maintain moisture content in cheese.
Milling	Curd pH = 5.35 Renneting to milling ~ 70 min	
Mixing	Curd temperature upon exit from mixer = 145 - 155°F	* lower fat curd is stiffer and it is important to maintain good curd pliability through the mixer - helps maintain high moisture and prevent fat losses into the mixer * higher mixer temperatures decrease microbial populations and inactivate residual milk coagulating enzymes

Table 5 describes key manufacturing steps in the manufacture of skim milk Mozzarella used in small scale production. This protocol is similar to that of reduced fat Mozzarella with the exception of the use of a fat mimetic and a curd rinsing step after milling. Without the use of a fat mimetic, the final moisture content of the skim milk Mozzarella is 59.5%. The moisture content with a carbohydrate-based fat mimitic (0.3% OptaGrade® 301) is 62-63%. A cold water rinse is necessary to help increase moisture content as well as maintain a final curd pH in the range of 5.220-5.25. The resulting skim milk Mozzarella cheese has good melt, stretch and chewiness characteristics, however the flavor is bland and upon baking the melted cheese forms plastic-like skin.

Table 5. Key manufacturing steps for the production of skim milk Mozzarella cheese.

Manufacturing Step	How	Why
Addition of fat mimetic	Add to cold raw milk	* increase cheese moisture content
Preacidification Addition of starter culture Firm milk coagulum Cooking Temperature Milling	Same as reduced fat Mozzarella cheese	
Cold water Soak	After milling soak curd in cold water for 20 minutes. Curd/water slurry temperature = 70°F	* increases cheese moisture content * helps to control final cheese pH (cold water rinse leaches lactose and lactic acid from the curd)
Mixing	Same as reduced-fat Mozzarella cheese	

Use of fat replacers in cheese

A fat replacer is a blanket term to describe any ingredient used to replace fat. There are four types of fat replacers (See Table 6), however in cheese, the use fat mimetics are most common. A fat mimetic requires a high water content to achieve its functionality and can be derived from proteins, carbohydrates and cellulose. Typically the protein - and carbohydrate- based fat mimetic particulate are smaller in size than the microcrystalline cellulose. The influence of fat mimetics on cheese physical properties is dependent on its size and how it associates with the protein matrix.

Fat replacers have been successfully used in a variety of cheese applications. Fat replacer should be flavorless, not adversely affect curd formation, not alter the rate of acid production and have minimal losses into the whey.

Table 6. Types of fat replacers.

Fat substitute	A synthetic compound designed to replace fat on a weight-to-weight basis, usually have a similar chemical structure to fat but resistant to hydrolysis by the digestive system.
Fat mimetic	A fat replacer that requires a high water content to achieve its functionality. ❋ protein-based (whey) - Simplesse®, Dairy Lo® ❋ starch-based (maltodextrin) - OptaGrade®, Stellar® ❋ fiber-based (soluble fiber) - OatTrim® ❋ fiber-based (microcrystalline cellulose) - Avicel®, Novagel® ❋ gums (methyl cellulose) - Methocel®
Low-calorie fat	A synthetic triglyceride combining unconventional fatty acids to the glycerol backbone which results in a reduced-calorie value
Fat extender	A fat replacement system containing a proportion of standard fats or oils combined with other ingredients.

Conclusion

In the development of manufacturing protocol for reduced fat and skim milk Mozzarella cheese it is important to understand the nutrient claims, so a correct target cheese composition (fat, moisture, protein, salt) can be established. Mozzarella physical characteristics are key to the performance of the cheese when used as an ingredient on pizza pies. Thus, in addition to compositional factors it is key that other chemical factors such as cheese pH and degree of casein hydrolysis be optimized.

Control of Microbial Contamination in Brining Systems

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Salting of cheese by immersion in brine is a common industry practice, especially for Italian cheese varieties. Salt contributes to flavor in cheeses, aids in whey draining, affects cheese texture and body, and also creates unfavorable conditions for growth of certain microorganisms by lowering water activity. In this workshop, practical aspects of brining systems are presented for control of pathogens. As a specific example of brining systems for the control of an undesirable pathogen, results are presented for the survival of *Listeria monocytogenes* inoculated to commercial cheese brines collected from cheese factories in Wisconsin and northern Illinois. Physical and chemical methods for elimination of *L. monocytogenes* from commercial brines are discussed.

I. Introduction

A. Roles of salt in cheese

1. Controls microbial growth and activity
2. Controls enzyme activities in cheese
3. Aids in whey drainage
4. Affects cheese texture and body
5. Influences flavor

II. Control of microbial growth

A. Useful terms

1. **Water activity** (a_w). Microorganisms require a certain quantity of available water for growth and survival. The water requirements of microorganisms is expressed as water activity:

   $a_w = p/p_0$ where p is the vapor pressure of the solution and p_0 is the vapor pressure of the solvent (usually water).

2. **Relative Humidity** (R.H.)

$$R.H. = 100 \times a_w$$

3. **% Brine**

$$\% \text{ Brine} = \% \text{ NaCl} \times 100 / \% H_2O + \% \text{ NaCl}$$

B. Minimum a_w values for microorganisms important in foods (adapted from Jay, 1996)

Table 3.5. Approximate Minimum a_w Values for Growth of Microorganisms Important in Foods

Organisms	a_w	Organisms	a_w
Groups		**Groups**	
Most spoilage bacteria	0.9	Halophilic bacteria	0.75
Most spoilage yeasts	0.88	Xerophilic molds	0.61
Most spoilage molds	0.80	Osmophilic yeasts	0.61
Specific Organisms		**Specific Organisms**	
Clostridium botulinum, type E	0.97	*Candida scottii*	0.92
Pseudomonas spp.	0.97	*Trichosporon pullulans*	0.91
Acinetobacter spp.	0.96	*Candida zeylanoides*	0.90
Escherichia coli	0.96	*Staphylococcus aureus*	0.86
Enterobacter aerogenes	0.95	*Alternaria citri*	0.84
Bacillus subtilis	0.95	*Penicillium patulum*	0.81
Clostridium botulinum, types A and B	0.94	*Aspergillus glaucus*[a]	0.70
Candida utilis	0.94	*Aspergillus conicus*	0.70
Vibrio parahaemolyticus	0.94	*Aspergillus echinulatus*	0.64
Botrytis cinerea	0.93	*Zygosaccharomyces rouxii*	0.62
Rhizopus stolonifer	0.93	*Xeromyces bisporus*	0.61
Mucor spinosus	0.93		

[a]Perfect stages of the *A. glaucus* group are found in the genus *Eurotium*.

C. Approximate water activities of various cheeses at the marketing stage.

Table 6. - Water activity of various cheeses at the marketing stage [41]

Cheese	a_w	Cheese	a_w
Brie	0.980	Gouda	0.950
Camembert	0.982	Gruyere	0.948
Cheddar	0.950	Minster	0.977
Cottage cheese	0.988	Saint Paulin	0.968
Edam	0.960	Parmesan	0.917
Emmental	0.972	Shrinz	0.940
Gorgonzola	0.970	Tilsit	0.962

III. Case example of control of an undesirable microorganism in cheese brines: *Listeria monocytogenes*

A. **Salt-resistant and other properties of *L. monocytogenes*.** *Listeria monocytogenes* is a halotolerant, gram-positive, facultatively anaerobic, nonsporeforming rod. The organism is capable of growth at temperatures as low as 1.1°C, (Juntilla et al., 1988), water activity as low as 0.90-0.92 (Nolan et al, 1992), and pH as low as 4.4 (Sorrells et

al., 1989). *L. monocytogenes* has been found in a variety of dairy products (Greenwood et al, 1991), and has been involved in several outbreaks of listeriosis involving dairy products (Farber and Peterkin, 1991).

B. Contamination of dairy plants and cheeses by *L. monocytogenes.*

L. monocytogenes is found more commonly in wet areas of dairy plants, such as floor drains, conveyers, floors, and equipment with condensate (Nelson, 1990; Rocourt and Cossart, 1997). The organism has been shown to attach to stainless steel surfaces at different pH and temperature levels in nutrient medium (Herald and Zottola, 1988). The brine system environment is humid and condensation is routinely present on product,equipment and building surfaces, posing a risk of cheese contamination with *L. monocytogenes.*

Evidence indicates a possible risk that cheese brines in a dairy plant could become contaminated with *L. monocytogenes*, either from environmental sources or by leaching of the organism from cheese contaminated with the pathogen (Ryser and Marth, 1991). The behavior of *Listeria monocytogenes* in commercial cheese brine systems is not well understood. Concerns include the possibility of survival of *L. monocytogenes* for extended periods of time in brines containing high salt levels, or increasing in numbers in brines with lower salt concentrations, such as those used for feta brines.

IV. Survival of *L. monocytogenes* in Commercial Brines

A. Commercial Brines and Composition

Thirty eight commercial brine samples were obtained from 14 cheese plants in Wisconsin and northern Illinois. Brines were kept refrigerated during transport to and storage at the Food Research Institute. Twenty six of the brines were from systems used to salt pasta filata varieties; mozzarella, string, provolone, fresh salami and giganti cheeses. One brine each was used to salt romano and parmesan. Seven brines were from systems used to salt both brick and Hispanic style cheeses. Three were from feta brine systems. One (F1) was obtained from a fresh brine storage tank and had not yet come into contact with cheese. Both raceway and static tank system brines were represented, as was a variety of filtration equipment. The proximate compositions of the brines were obtained.

B. Survival in Commercial Brines

The survival of *L. monocytogenes* varied considerably depending on the commercial brine sample. Survival ranged from less than 7 days to 259 days, the last sampling time. No correlations were noted among length of *L. monocytogenes* survival and microbial populations, mineral content, nitrogen content, or type of filtration systems used. Temperature influenced the survival of *L. monocytogenes.*

V. Methods to Conrol *L. monocytogenes* in Cheese Brines

A. **Filtration.** It is known that certain filtration systems are capable of removing pathogens and spoilage microorganisms from cheese brines. However, many of these systems are expensive and may not be cost-effective for smaller cheese manufacturers.

B. Antimicrobials. **Several antimicrobials were evaluated for control of *L.***

***monocytogenes*. Among those tested, low concentrations of hydrogen peroxide and sodium hypochlorite were effective in eradicating *L. monocytogenes*.**

VI. Conclusions and Recommendations

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A Dynamic Model to Explain Mozzarella Cheese Appearance and Functionality During Pizza Baking

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INTRODUCTION

Given the healthier eating goals of consumers and the continued demand for pizza there has been an interest in developing a low fat Mozzarella cheese (1-8). Rudan et al. (5) and other investigators have observed that reduction in fat content of Mozzarella cheese causes changes in overall cheese composition, proteolysis during refrigerated storage, and changed the pizza bake performance. High fat cheeses (ca. 15 to 25% fat) have better melting and browning compared characteristics to low fat cheeses (ca. 5 to 10% fat). The common interpretation of these results has been: a reduction in fat, equals a reduction in functionality. However, there are at least three other major characteristics of Mozzarella cheese that change as a result of fat reduction: 1) lower ratio of moisture to protein (i.e., lower moisture in the nonfat substance), 2) lower amount of proteolysis, and 3) a lower amount of free oil release.

Fife et al. (1) and Merrill et. al. (3) investigated the effect of moisture to protein ratio on functional properties of low fat Mozzarella cheese. By increasing the moisture to protein ratio, they (1,,3) found some improvement in melted cheese functionality, however, they did not report any pizza bake data. Tunick et al. (7) increased the amount of proteolysis prior to pizza baking by storing low fat Mozzarella cheese at refrigeration temperature for 70 days (much longer than normal). They reported improved pizza bake functionality, however, under heating conditions greater than 175 C the cheese "browned heavily". Because many pizza restaurants bake pizza at temperatures $\geq$ 260 C, the tendency of cheese to brown excessively has become a particular concern to the Mozzarella industry (2). Thus , proteolysis alone does not seem to be the key difference causing the poor melting and browning characteristics of low fat Mozzarella cheese. To date there have been no studies on the third major difference between full fat and low fat or fat free Mozzarella cheese, namely the free oil release.

For low fat Mozzarella cheese mentioned above, the free oil results seemed to be directly related to melted cheese functionality (i.e., more free oil release better melting and browning behavior) during pizza baking (5). In a separate study (4), the control cheese which released significantly more fat, performed better during pizza baking compared to cheeses made from homogenized milk or cream. Could the small amount of free oil on the surface of the cheese shred determine the melting and browning of the Mozzarella cheese during pizza baking and if so, how could this help to explain cheese melting and browning?

Our objective was determine the effect of free oil release on fat free, low fat, and full fat Mozzarella cheese functionality during baking. The effect of a physical barrier on fat free, low fat,

and full fat Mozzarella cheese functionality was also determined. Based on these results a dynamic Mozzarella cheese melting and browning model is proposed.

MATERIALS AND METHODS

Simulated Pizza Bake Test

Three cheeses of different composition were used in the simulated pizza bake test: 1) commercial fat free (<1.0% fat, 64.2% moisture, and 27.4% protein), 2) Cornell pilot plant produced low fat (5.8% fat, 54.0% moisture, and 32.1% protein), and 3) commercial LMPS Mozzarella cheese (21.0% fat, 49.1% moisture, and 25.8% protein). Shredded cheese (ca. 100 g weighed to the nearest 0.01 g) was spread evenly on the bottom of a 20.3 cm diameter x 3.2 cm deep pre-weighed aluminum pan. The cheese in the pan was then baked in an impinger pizza oven (Impinger Model 1132, Lincoln Food Service Products, Inc., Fort Wayne, IN) for 5 min and 232 C. There were three different treatments: 1) nothing was done to the pan and cheese (control), 2) about 1 g of vegetable oil (100% canola oil, Commander Foods, Inc., Syracuse, NY) was sprayed onto the surface of the cheese shreds after they were placed in the pie (hydrophobic surface coated, **HSC**), and 3) aluminum foil was placed over the aluminum pan and cheese (physically covered, **PC**). The HSC was used to simulate the effects of fat release during pizza baking. A hand pump spray bottle was used to spray the oil on the cheese surface. The average amount of vegetable oil sprayed on the fat free, low fat, and LMPS Mozzarella cheese was, 0.84 g, 0.80 g, and 0.99 g/100 g of cheese, respectively. After baking the cheese was allowed to cool to room temperature (ca. 5 min) and re-weighed to determine the amount of moisture lost. Moisture loss was divided by the total initial weight of cheese, multiplied by 100, to express the moisture loss per 100 g of cheese. Pictures of the cheeses were taken after baking to record shred fusion, melting and browning of the cheese. The simulated pizza bake test was done to eliminate any effects of the sauce or the pizza dough on the results.

Actual Pizza Bake Test

The actual pizza bake test was used to confirm (simulated test results) melting and browning behavior of the cheese when used as a topping for pizza. The pizza was made by placing 150 g of tomato sauce (Ragu Traditional Old World Style, Van Den Bergh Foods Co., Lisle IL) on a 30 cm pizza crust (Frozen Deli Style, P&C Food Markets, Inc., Syracuse, NY). Cheese and HSC were applied as described below. All pizzas were baked using the same conditions as the simulated pizza bake test.

Commercial fat free Mozzarella cheese was shredded and 300 g was placed over the sauce and crust. Prior to baking, one half of the pizza was temporarily covered with a plastic film to prevent that half of the cheese on the pizza from being sprayed, while the other half was sprayed with Pam® (Butter Flavor, American Home Food Products, Inc., Madison, NJ) to form a hydrophobic surface coating on the exposed surface of the cheese shreds on top of the pizza. The total spray time was about 4 seconds which corresponded to about 3 g of coating material for 150 g of cheese. The exact amount of hydrophobic surface coating material applied to the cheese in the trial is not known, because in addition to canola oil, Pam® contains other ingredients. Pam® was used instead of the hand pump dispenser containing vegetable oil because the aerosol spray delivery system for the Pam® gave a produced a more uniform dispersion of the hydrophobic surface coating on the cheese

shreds.

RESULTS AND DISCUSSION

Simulated Pizza Bake

The results of the simulated pizza bake test are shown in Table 1. It was thought that the HSC might reduce total moisture loss, however this was not the case. For any given fat level, the amount of moisture loss for the control and HSC cheeses was about the same, while the PC cheeses lost much less moisture. The overall water loss at any fat level was surprisingly large; it was not expected that Mozzarella cheese would lose about half of its moisture when baked under the conditions used for baking pizza. Given such a large moisture loss, it would be expected that the functional properties of the cheese on the pizza, after baking, are significantly different compared to the functional properties of the cheese before baking. However, there are reports in the literature comparing functional properties of Mozzarella cheese before and after pizza baking.

Even though moisture loss from the HSC cheese was the same or greater than the control, the effect of the HSC on melting and browning for the fat free and low fat cheese was dramatic. The entire surface of the control cheese formed a skin and raised about 5 cm upon baking, followed by scorching of the raised cheese surface. The low fat and fat free cheese shreds treated with a HSC melted, fused, and formed light brown colored blisters similar to those normally found when using cheese with much higher fat. These results indicated that the moisture lost **at the surface** of the cheese shred and the subsequent skin formation on the surface of the cheese shred was more important and not the overall moisture loss. The control and HSC cheese for the LMPS Mozzarella had the same melting and browning. This was not surprising because the higher fat LMPS Mozzarella cheese inherently has enough free oil release to prevent surface moisture loss and skin formation upon baking. Therefore, the added free oil of the HSC treatment had no effect on the LMPS Mozzarella cheese melting and browning.

At any given fat level, the PC cheese lost about half the moisture that was lost by the control and HSC cheeses. For the fat free, low fat, and LMPS Mozzarella cheese this resulted in complete cheese shred melt, but no browning. The lack of browning could have been due to the high relative humidity at the cheese surface during baking. However, more work is needed in this area to clearly identify the effect of PC on the behavior of cheese during baking. In any case, covering the cheese surface with a hydrophobic barrier (applied or inherently present) or a physical barrier has a dramatic effect on fat free, low fat, and LMPS Mozzarella cheese melting and browning.

Actual Pizza Bake

Commercial fat free Mozzarella cheese lightly coated with Pam® completely melted and formed small light brown blisters like that of LMPS Mozzarella cheese during pizza baking (upper half, Figure 1). The untreated fat free cheese (lower half, Figure 1) had limited shred melt and fusion, and excessive browning and scorching. The results for low fat Mozzarella cheese (not shown) were the same. As stated in the above section, a light hydrophobic surface coating (ca. 2% Pam®) prevented cheese shred surface moisture loss and subsequent skin formation. The results clearly show that fat free and low fat Mozzarella cheese can have acceptable melting and browning on pizza.

Model of the Dynamics of Melting and Browning

Based on the results from this study it appears that fat, moisture, and protein all play a role during Mozzarella cheese melting and browning. In addition, the time series and locations of events taking place on the surface of the pizza are important. Our model describes proper melting and browning for a LMPS (full fat) Mozzarella cheese and the reasons why fat free and low fat Mozzarella cheese do not have these desirable characteristics. Starting from frozen or refrigerated temperature, first, the fat melts (complete at 38 C) and then it expands as temperature increases. Expansion of the melted fat forces it to move out of the protein matrix and on to the cheese shred surface. As the cheese temperature increases to 50 to 80 C, the molecular vibration within the cheese increases and casein matrix interactions with other components change causing the cheese structure within a shred to no longer support its own weight and the cheese starts to deform and flow due to gravity. At the same time, temperature dependent interactions between the casein matrix, components dissolved in the water phase, and calcium cause an increase in whiteness of the cheese on the pizza. These temperature induced changes in whiteness are reversible and the cheese will decrease in whiteness as it cools after baking.

It is absolutely critical that the surface of the shred not dry to the point of making a hard shell that prevents the cheese inside the shred from flowing when the internal temperature of the shred reaches the temperature at which it starts to flow due to gravity. Release of free oil onto the surface of the cheese shred to protect the surface from dehydration is the critical event that produces proper melting behavior during baking.

Above 100 C (at the cheese surface and below) water is converted to steam and this steam plus trapped air between cheese shreds collects as bubbles under the molten cheese surface. Next, the cheese surface begins to rise due to the expansion of steam and trapped air. In certain areas the cheese rises and the formation of a blister begins. The size of the blister will be dependent on the size of the bubble beneath the cheese surface and, with respect to the cheese characteristics, the amount of expressible serum, the amount of proteolysis, and moisture to protein ratio, which all influence the apparent viscosity of the cheese. That is, the greater the capacity of the cheese to stretch without breaking as the gas bubble tries to push up through the molten cheese, the larger the blister. As the cheese rises, the top of the cheese becomes thin, liquid fat flows down the sides of the forming blister, moisture is lost from the thin film of cheese at the top of the blister, and the top of the blister dries and turns brown. Where sufficient free oil is still present and moisture is not lost the cheese remains white in appearance. The areas with different appearance on the surface of a pizza differ only in the degree of surface moisture loss. This explains why a Mozzarella cheese with a homogeneous lactose or galactose content can have a heterogeneous appearance (localized browning) during pizza baking. This series of events leads to the overall appearance of LMPS cheese on pizza.

In fat free or low fat cheese there is a lack of sufficient free oil release on the surface of the cheese shred at low temperature (i.e. < 50 C), thus at higher temperatures the cheese surface rapidly dehydrates. As a result a brown skin or shell forms on the surface of each shred before the center of the shred reaches a temperature sufficient for flow and deformation of the shred structure due to gravity. Therefore, the fat free or low fat cheese does not flow and fuse with adjacent shreds. Further dehydration leads to excessive browning and scorching of the surface of individual shreds giving the pizza a dark brown appearance with many dark brown intact shreds present.

After lightly coating a low fat or fat free cheese shred surface with a small amount of hydrophobic material to prevent the surface dehydration during pizza baking, the melting and browning of fat free, low fat, and LMPS Mozzarella cheeses are similar. Furthermore, only a small amount of hydrophobic material, present early in the baking process, is required.

In summary, fat migration to the shred surface, which prevents rapid dehydration, is the critical event limiting melting and controlling browning of fat free and low fat Mozzarella cheese during pizza baking. A barrier to block moisture loss using a thin hydrophobic surface coating of the shred produces excellent melting and browning of fat free and low fat Mozzarella cheese during pizza baking.

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Table 1. Moisture loss (g/100g cheese) from fat free (0% fat), low fat (5.8% fat), and LMPS[1] (21% fat) Mozzarella cheese shreds that were not covered (control), hydrophobic surface coated (HSC), and physically covered (PC).

Cheese	Treatment[2]			SEM	LSD[3]
	Control	HSC	PC		
Moisture Loss					
Fat Free	32.04[a]	31.58[a]	15.91[b]	0.54	1.73
Low Fat	28.23[b]	29.25[a]	15.46[c]	0.17	0.56
LMPS	27.92[a]	27.30[a]	15.39[b]	0.25	0.81

[a,b,c] Means within same row not sharing common superscripts are different ($P < 0.05$).

[1] Low Moisture Part-Skim

[2] n = 3

[3] $P = 0.05$.

Figure 1. Appearance of a pizza topped with commercial fat free Mozzarella cheese after baking (Impinger oven, 232 C, 5 min) with no hydrophobic surface coating (lower half) and with hydrophobic surface coating (Pam®, upper half).

Potential Off-Flavors in Cheeses Caused by Brine Salting

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Sodium chloride exerts notable influences on the flavor properties of cheeses, including both direct and indirect contributions. Many recent experiences with defective flavors and textures resulting from attempts to produce no-salt cheeses have reinforced the recognition by the consumer and cheesemaker of the importance of salt in cheese. Insufficient salt, either by brining or direct salting, in aged cheeses permits overly active growth of non-starter lactic acid bacteria and other adventitious organisms that readily leads to the development of a variety of unclean flavors. Inadequate penetration of brine can lead to such excessive proteolysis and microbial activity in the center of cheeses, such as blue cheese, that the cheeses are marginally salable.

Contamination of cheeses by wild populations of microorganisms from brine tanks and related equipment frequently leads to the development of off-flavor compounds in cheeses that are subsequently held or aged notably. Partial metabolism of certain aromatic amino acids by these bacteria is often the chemical source of these flavor defects.

Chemical or phenolic taints occur in brine-salted cheeses where the presence of UV light and sodium hypochlorite (or hydrogen peroxide) used to control contamination produce conditions leading to the bromination of naturally-derived p-cresol. Such taints are generally very offensive, and the origin has sometimes have been difficult to determine. However, elimination of either the UV light or the sodium hypochlorite prevents the formation of the taint compounds.

Impact of Brine Quality and Salting on Potential Flavor Defects in Cheese

1. **Overview of the role of salt in the control of cheese flavor development**

2. **Flavor defects in cheeses caused by microbiologically contaminated brine and related equipment**

3. **Secondary flavor defects caused by attempts to control microbial contamination in brining operations**

Impact of Salt on Cheese Flavor Development

—Low-salt cheese experiences have clearly shown the consequences of inadequate salt on cheese flavor quality

—Besides contributing to the flavor directly, salt:

—Inhibits over-activity of cheese ripening bacteria
—Controls the activity of proteolytic enzymes
—Affects water binding properties of proteins

Impact of Salt on Cheese Flavor Development

—In Cheddar cheese, salt-in-moisture (S/M) provides a good indicator for projecting cheese quality

—S/M values of 4.7-5.7 are associated with optimum cheese quality

—S/M values that are lower (S/M <4) accelerate proteolysis and fermentations while higher S/M values (>6) retard flavor and texture development

Impact of Salt on Cheese Flavor Development

—Low S/M (<4) in cheeses often leads to unclean flavor defects during aging

—Unclean flavors result from aromatic amino acid degradations by certain non-starter lactic acid bacteria

—Examples include the flavors of indole from tryptophan and phenethanol from phenylalanine

Impact of Salt on Cheese Flavor Development

—The method of salting, i.e., dry-salting of curds, dry-salting of cheeses or brining, lead to specific problems

—Lack of uniformity of salt uptake from brine often leads to defects

—Several factors, including brine strength, cheese pH, and temperature can be involved

—Careful control of cheese manufacture and brine needed

Impact of Salt on Cheese Flavor Development

—Inadequate penetration of salt into dry- and brine-salted cheeses leads can lead to defects

—Lack of salt in cheese centers allows excessive microbial and enzymic activitities, causing soft centers and sometimes pronounced unclean flavor defects

—For blue cheeses and slime-ripened cheeses, salt content is an important determinant in establishment of ripening microflora, and the character of the resulting flavors

Defects in Cheeses Caused by Microbiological Contamination of Brine and Related Equipment

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—Brine is a defining source for the characteristic microflora of cheese plants with brining operations

—Important flavor-related groups are many yeasts, non-starter lactic acid bacteria, coryneforms & Brevibacterium linens, Geotrichum candidum, and a wide range of contaminants

—Microbial defect flavors aren't usually picked up directly from "dirty" brine

Defects in Cheeses Caused by Microbiological Contamination of Brine and Related Equipment

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—Most brine-related off-flavors result from growth of microbes on cheese surfaces during aging and handling

—Microbial growth on the rind may appear as a slime, especially when excess moisture is present

—Rinsing excess salt brine from cheese helps prevent hygroscopic moisture accumulation and subsequent slime formation

Defects in Cheeses Caused by Microbiological Contamination of Brine and Related Equipment

—Yeasty flavors are the most common off-flavors directly associated with brined cheeses

—Yeasty flavors are caused primarily by yeast metabolism of amino acids to corresponding flavor compounds

—The main yeasty flavor compounds are the 'fusel oil alcohols' that are noticeable in alcohol and bread

Defects in Cheeses Caused by Microbiological Contamination of Brine and Related Equipment

—Brine contamination-derived flavors can show up at a later date in cut or shredded cheeses

—Often these flavor defects develop once the cheese is in the consumers' possession

—The solution to brine contamination flavor defects is maintenence of clean brine solutions and good sanitation of the brining facility

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

Kerosene-like Flavor Taints

—Potassium sorbate has been added to brine in attempts to suppress microbial contamination

—Sorbate is most effective in controlling molds, but it has significant suppressing effects on yeasts and bacteria

—However, sorbate usage in this application can lead to significant flavor problems

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

—Sorbate stresses molds, and they detoxify it by converting it to pentadiene

—Pentadiene is the "kersosene" tainting compound, and it is very penetrating and unpleasant

—Pentadiene taint usually develops when mold is attempting to grow at some later time, often in shredded cheeses

—Pentadiene tainting is not restricted to brined cheeses

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

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Chemical, Medicinal, and Phenolic Taints

—Relatively common flavor defects in brined cheeses

—Chemically formed in brines and on cheese surfaces

—Key activators are UV light, sodium hypochlorite, or hydrogen peroxide

—Taint compounds are bromophenols

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

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Chemical, Medicinal, and Phenolic Taints

—Phenols have relatively low medicinal flavors, but brominated phenols extremely potent flavors

—Medicinal, phenolic flavor taints are very persistent and unpleasant

—In addition to p-cresol in cheese, also find natural phenol and other alkyl phenols that can participate n the bromination reaction

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

Prevention of Bromophenol Medicinal Taints

—Can't do much about natural phenols present in cheese

—Phenols can be provided from other sources

—Sea salt has high levels of bromide salts, while more refined salts have lower levels

—Most direct control is to eliminate the use of UV lights, sodium hypochlorite, and hydrogen peroxide in brining

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

Chemical, Medicinal, and Phenolic Taints

—Chlorophenol formation resulting from reactions between phenol and hypochlorite sanitizers

—Different from bromophenol formation, but yields medicinal flavors also

—Documented occurrences in brined cheeses are very sketchy; May require higher concentrations to cause taints than bromophenols

Secondary Flavor Defects in Brined Cheeses Caused by Attempts to Control Microbes

Other Derived Flavor Taints: Mustiness

—A number of molds can modify the halogenated phenols (bromo- and chloro-) to form corresponding anisoles in a detoxification mechanism

—Chloroanisoles are extremely musty compounds that are among the most potent flavor compounds known

—Suspected to be present in some cheeses, but are very difficult to analytically verify their presence

Cheese Grading, A More Practical Approach

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INTRODUCTION

Acid, Bitter, Unclean, Whey Taint, Fermented, Short, Weak, Pasty, Open, Slitty, Gassy, Mottled, Acid Spots, the defects of cheese go on and on. Complicating the defined defect when evaluating cheese is the decision of the grader as to the severity of the defect, very slight, slight, definite and pronounced. Is there no wonder that cheese grading or evaluation is a very subjective process? The ability of a cheese grader to properly identify a defect is an important aide to help determine potential cheese manufacturing problems when cheese "does not make the grade". Cheese grading should not be taken for granted within a cheese production plant. In fact, a good Cheese Grader and Dairy Scientist should be among the most regarded personnel in your cheese plant. Fixing a quality related problem is much easier when a defect is properly identified. What does this mean? Acceptance of a cheese as either an acceptable product at a premium price or a down-graded product at a much reduced price is at stake. The sooner a cheese defect is identified, the sooner you can get back to manufacturing a quality product.

Who is the judge of quality? Is it important to meet the rigorous scrutiny of the USDA standards for sale to the government? Is it the customer looking for consistency from one cheese supply and another? Is it your own personal standards? Is it a Judge at a Local, Regional, State or International Cheese Competition? Or, is it the end-use customer, the consuming public?

Cheese grading is a talent. Cheese grading is not an "Art" or a "Science". All cheese "Graders" are self proclaimed "Experts"! A very confident group of individuals. How does one become a cheese grader? Easy, get a trier, put on a white lab coat, tell the producer you are the grader, plug the cheese and finally tell the customer you are going to accept or reject the cheese sample. Some states, Wisconsin for example, say you must have a license to do this. Once you have a license in Wisconsin you are a grader.

Many cheese graders comment, "I might not be right, but I am official"! Well, in a contest that is very true. However, if your interest is not focused on winning the World Cheese Contest but rather targeted to make a product acceptable to the consuming public, day in and day out, a more practical approach to cheese grading is necessary.

PRESENTATION

It is important to recognize your Cheese Grader as a KEY Employee. Unfortunately, there is no formal training program available for training your employee. Many times a Cheese Grader is selected as an individual having the most "Experience" in the Dairy Industry.

Anyone can be an effective Cheese Grader. It may be an individual within your organization you would least likely expect! The USDA holds many clinics throughout the year to evaluate cheese samples. If you have attended one of these clinics they are very informative and allow interaction among a wide cross section of graders to compare notes and attempt to unify grading expertise. However, these clinics do not identify the potential an individual has to evaluate cheese. Perhaps you are not sending the right people to these clinics.

Every individual has varying responses to the basic senses of touch, sight, sound, taste and smell. Cheese grading deals with all of these five basic senses, touch, sight, taste and smell. However, the most critical of these senses are taste and smell. Each of us not only have the ability to taste and smell but our detection or threshold level to identify various flavors and aromas differ greatly.

Selecting a gifted individual for evaluation of a Dairy Product can be much easier than you think. It is easy to prepare "mock" flavors and various aromas typical of flavor and aroma attributes of cheese.

FLAVOR

The human tongue is able to sense four basic sensations, these sensations are acid, bitter, sweetness and salt. Each of these sensations stimulate a different part of your mouth and tongue.

Selecting individuals capable of identifying these basic sensations is very easy! Mild solutions of a non-volatile acid, quinine water, sodium chloride and lactose are easily prepared. For demonstration purposes, to accompany this presentation, four solutions have been prepared for evaluation. Additionally, two solutions have been prepared using lower concentrations of to serve as an unknown. The four basic solutions were prepared as follows:

Sensation	Water (ml.)	Ingredient	% Solution
Sweet	100	0.5 g. Lactose	0.5%
Acid (Sour)	100	.15 ml. 20° Baume HCl	0.15%
Bitter	100	2 ml. Tonic Water	2%
Salt	100	0.5 g. Sodium Chloride	0.5%

Smell each of these solutions. Record your results. Taste each of these solutions. Make note where in your mouth you identify the flavor sensation. Record your results. These samples should not have any aroma. However, they should be easily detected by the discriminatory palette. If your taste buds are not stimulated by the prepared solutions you should probably not consider grading cheese.

Two "unknown" samples have been prepared. Smell each solution. Record your results. Taste each solution. Record your results. Make note where in your mouth you identify the flavor sensation.

Sensation	Perceived Flavor
Acid	Sides and Back of Tongue
Bitter	Tip and Back of Tongue
Salt	Middle of Tongue
Sweet	Tip and Sides of Tongue

To determine a threshold level or level of sensitivity you can further dilute these solutions until undetectable. How low can you go? Who in your organization has the most ability to distinguish each of these basic sensations?

Again, it is very important to determine sensations specific to an individuals perception. This is why Grading or Evaluation of Dairy Products is considered "SUBJECTIVE". However, it is extremely important to associate pre-prepared samples and their sensations to each specific individual and as mentioned earlier, to determine the sensitivity or threshold of that individual.

AROMA

Just as important to identifying flavor defects, the need to identify aromatic or volatile defects is essential. Where many flavor defects are not volatile, all of the volatile defects in cheese also stimulate the sense of taste. Also, similar to flavor defects, aromatic defects can be simulated to determine a graders ability to establish a response to the defect as well as establish a threshold level for the specific defect.

For demonstration purposes, to accompany this presentation, four solutions have been prepared for evaluation. Additionally, two solutions have been prepared using lower concentrations of to serve as an unknown. The four basic solutions were prepared as follows:

Sensation	Milk (ml.)	Ingredient	% Solution
Rancid	100	Calf Lipase	0.5%
Fermented	100	6 Parts Pineapple Juice 1 Part Vinegar 1 Part Apple Juice 1 Part Grape Juice	1%
Yeasty	100	Bakers Yeast Solution	1%
Whey Taint	100	Acid Whey	2%

Smell each of these solutions. Record your results. Taste each of these solutions. Make note where in your mouth you identify the flavor sensation. Record your results. These samples should be very aromatic. Like flavor detection, if you cannot taste or smell the prepared solutions at the presented level, you should probably let someone else do your product evaluation.

Two "unknown" samples have been prepared. Smell each solution. Record your results. Taste each solution. Record your results.

We have had the opportunity to experience 4 flavor and 4 aroma defects associated with cheese. Most of the common defects can be synthesized to help educate, select or "tune" up your graders. The levels that have been presented should be easily detected. The next step is "fine-tuning" or

selecting the most sensitive palette within your quality assurance department. Use the starting concentrations discussed and dilute the samples in small increments until a threshold level has been determined (a point at which an individual cannot distinguish the diluted sample from a control blank). You have now found the best "grader" in your operation. Who is it?

While flavor and aroma are probably most apparent to the consumer, body and texture are extremely important to the secondary manufacture when preparing cheese for final use. This is where the senses of touch and sight play a significant role.

BODY (Touch)

Body plays a very important role to the secondary processor and consumer alike. The secondary processor finds body and texture to play a significant role in efficiency of manufacturing. Unlike Flavor and Aroma detection, the physical defects to be discussed cannot be prepared or "mocked-up". The importance of defects determined by mechanical inspection are much more objective than the flavor and aroma defects previously discussed.

Body of cheese is determined by our sense of touch. Body defects of cheese differ greatly among varieties opposed to flavors and aromas. For example; Blue cheese may be expected to be crumbly and short; Brie and Camembert might be expected to be weak and pasty; aged Parmesan better be firm and corky; aged Cheddar Cheese may be expected to be slightly short.

Experience tells us that these defects might be less criticized by the consumer but cause great problems to the cheese processor or packager. Body of the cheese plays and extremely important role in machinability. A short, crumbly, curdy, corky or mealy defect would make slicing or cutting cheese into smaller retail pieces very difficult and result in inefficiencies and product losses. A weak or pasty defect would make shredding as well as cutting and slicing a difficult task.

Most cheeses should have a firm body that resists compression when pressed between the thumb and forefinger. When compressed between the fingers it should spring back. When removed from the cheese trier the cheese should be flexible when bent and resist a sharp snap when bent. Desired body of a cheese plug would possess a nice clean tear when bent.

TEXTURE, COLOR and APPERANCE (Sight)

Texture, Color and Appearance all play a significant roles in the perception of quality to the consumer, packager/processor and cheese grader.

In the eyes of the consumer, a rough surface, bleached (oxidized or pinking), open texture indicates something is wrong with the cheese. This is especially true when displayed in the Dairy Case next to a selection of cheese having no visual defects. Acid spots, mixed or dried curd, fines, mottled and light color are also defects that would lead the consumer to make another selection.

In the eyes of the packager/processor, an open piece of cheese creates a situation where cheese density could effect the weight of the cheese when cut into smaller retail pieces, resulting in short

weight packages. A misshapen block of cheese would result in excessive trim loss. Cheese with mixed curd, fines, mottledness or waviness would slow down production lines and also result in cheese losses.

The packager/processor looks for uniformity. Their specifications for color are important to allow for a defined profile or consistency of appearance in the Dairy Case.

The significance to the Cheese Grader when evaluation cheese, especially in Contest situations, brings a new meaning to quality. Many times the difference of the "Grand Champion" and the "Runner-up" or "Reserve Champion" is block appearance or integrity. A Cheese Judge or Grader looks very carefully at final workmanship of the finished product. Lopsided, wrinkled package, misshapen block, dished surface are critically examined in contest situations. If your intention, for the sake of attending this presentation, is to "win the contest", make sure your entry is wrinkle free, has sharp square corners and the style has the proper shape and exterior integrity.

THE CHEESE GRADER

The Cheese Grader is not the enemy, the Cheese Grader is an ally. We have discussed how to train your in-house grader by preparing "mock-up" samples. Now, what defects should be critiqued? Following is a sample Cheddar or American Cheese Grade Sheet.

CHEDDAR CHEESE GRADE SHEET

ENTRY NUMBER	
CLASS	

DEFECT KEY

Very Slight	Detected under very critical examination	0.1-0.5
Slight	Detected under critical examination	0.6-1.5
Definite	Easily detected	1.6-2.5
Pronounced	Easily detected, intense	2.6 +

DEFECT	FLAVOR DEFECTS (45 POINTS)				SCORE
	V. Slight	Slight	Definite	Pronounced	
Acid					
Bitter					
Fermented					
Flat					
Fruity					
Rancid					
High Salt					
Sulfide					
Unclean					FLAVOR SCORE
Yeasty					
Other					
BODY and TEXTURE (30 POINTS)					
Corky					
Curdy					
Crumbly					
Mealy					
Open					
Gassy					
Pasty					
Short					
Weak Sweet Holes					BODY & TEXTURE SCORE
Pin Holes					
Other					
APPEARANCE (15 POINTS)					
Foreign Material					
Huffed					
Mold Under Wrap					
Surface Mold					
Irregular Surface					APPEARANCE SCORE
Wrinkled Wrap					
Other					
COLOR (10 POINTS)					
Acid Cut					
Unnatural					
Mottled					
Wavy					
Seamy					COLOR SCORE
Mixed/Dried Curd					
Other					

JUDGE SIGNATURE: _____

DATE: _____

TOTAL SCORE	

A score card as presented above is used during a contest situation but seldom used for general everyday cheese grading. This is where cheese Grading and Cheese Scoring take different paths. If you want to win a contest, scoring is extremely important criteria. However, if you are just trying to satisfy the general consumer an opinion of acceptance is adequate. Where scoring splits hairs to find a victor, grading simply put, evaluates general acceptance.

For a cheese to win a contest you must strive for the perfect score, 100. However, minor defects are allowed for meeting consumer acceptance. If a score tally was made for your daily production, a number 1 cheese, USDA Grade A Cheese would allow a score between 92 and 100. One can liken a contest compared to general production by shooting at a dart board. Contest cheese better shoot for the bulls-eye, cheese for general manufacture can be allowed in the outer ring!

Cheese Grading standards and guidelines commonly used for grading are published by the United States Department of Agriculture publication Section 58.2501-2506 and Wisconsin Department of Agriculture publication Chapter ATCP 81, Cheese Grading, Packaging and Labeling standards.

DEFECTS, CAUSES and CURES

Once a defect is properly identified it becomes much easier to relate to procedural changes to correct the cause. Some of the common defects and their probable cause are listed below.

Defect	Cause	Solution
Acid	Bacteria, contamination or added	Culture selection, short wash HTST during long runs
Bitter	Culture selection, coagulant selection, contamination, low salt in moisture	Culture selection, short wash HTST during long runs, check salt/moisture
Fruity/Fermented	Heterofermentative Lactobacilli	Check cooling rate, short wash HTST during long runs
Unclean/Utensil	Contaminating bacteria	Sanitize all equipment, check wash tank sanitizer level, enforce personal hygiene
Rancid	Milk conditions or treatment of milk fat or possible bacterial	check raw milk, watch for warm milk, watch for over aeration and agitation
Yeasty	Yeast contamination	Clean equipment, check sanitizer levels, check milk
High Salt	Salt	Measure salt and cheese yield
Sulfide	Bacterial Contamination or milk heat treatment	Check for excessive heat treatments, bacterial contamination
Whey Taint	Entrapped whey	Allow for proper whey drainage, don't press too hard too soon, insure adequate cook procedure
Curdy	Lack of acid development, too much salt	Check calcium chloride level, salt level, acid development and moisture content
Open	Entrapped whey, improper pressing	Encourage slow pressing with gradual increase in pressure
Short	Acid development, pH	Too much early acid development, check for low salt and low moisture
Mealy	Acid development, contaminating bacteria, coagulant	Too much early acid development, check for low salt and low moisture
Weak	Moisture, culture selection, salt in moisture	Proteolytic contaminant, high moisture, low salt/moisture
Pasty	Moisture, culture selection, salt in moisture, bacteria contaminant	Proteolytic contaminant, high moisture, low salt/moisture
Slitty	Spore forming bacteria contaminant	Short wash HTST during long runs, check raw milk supply, check yeast and heterofermentative lactobacilli
Gassy	Gas forming contaminant	Check coliform, yeast and heterofermentative lactobacilli. Check milk, sanitation, CIP and personal hygiene
Corky	Coagulant, lack of pH development, Calcium abundance	Check proper pH carry-through, reduce calcium chloride, increase coagulant
Seamy	Minerals on curd surface	Keep curds warm at all times,

		rinse curds, allow proper mellow time for salt to dissolve, don't over stir curds
Wavy	Fat disturbance, whey cream or over-stirred set	Check cheese set, do not over stir set, make sure no eddy's when agitator is shut off, proper dilution of coagulant, don't over-stir curds
Acid Spots	Make procedure, cooling	Check for matting curd, proper agitation during cook, slow cook to allow proper syneresis, even salt distribution
Mottled	Make Procedure, cooling	Check for matting curd, proper agitation during cook, slow cook to allow proper syneresis, even salt distribution
Bleached Surface	Oxidation, packaging	Sufficient color, fresh color, tight packaging

CONCLUSION

Cheese Grading is subjective. While subjective, methods have been presented to help identify how to best select a cheese grader relative to the ability to determine or distinguish flavors and aromas through "mock-up" or pre-prepared sampling.

We have many graders to contend with; the Scoring Judge, the Packager/Processor and the Consumer. Each "Grader" has a different criteria of acceptance. Our challenge is to satisfy all graders or critics.

A good cheese grader uses all senses when evaluating cheese. By using our senses and rely on our evaluation we have learned that we can relate these grading observations to our make procedure and make crucial decisions to identify causal problems.

The five basic senses were discussed at the onset of this presentation. The sense of sound has not been mentioned. The most satisfying of our senses is sound. When the consumer says mmmmmmmmm! Our mission has been accomplished. We have satisfied taste, smell, touch, sight and sound. If we have not paid attention to our abilities to properly identify defects, our sense of sound ugggghhh! will be directed to our manufacturing department. Good luck graders!

Salting Alternatives and Mozzarella Productivity

Jim Fischer
Basic Concepts, Inc

In the Good Old Days . . .

In the good old days, I am told, adding salt to mozzarella was a far simpler matter. There was enough time to keep mozzarella in the brine long enough to get all the salt we needed. With competitive pressures, more and more emphasis was placed on getting as much

product out the door as quickly as possible. The problems from pre-salting just had to be dealt with somehow; because the important thing was increasing productivity and meeting the demands of the customers and the accounting department.

Today the costs of disposal, the high cost of installing and maintaining filtration and other systems, and environmental pressures have caused many producers to take a hard look at how they will cope with salt waste challenges today - and down the road.

Overview

It is a given that salt is a necessary ingredient for quality mozza. Its contributions are considerable; controlling Ph. , its benefits to flavor, and it influences on the overall control of the chemical process that makes up what is known as mozza. The difficulty lies in the almost total lack of efficiency in getting the salt into the product on a consistent basis. In the average mozza plant in the United States, it is estimated that almost 60,000 pounds of salt are wasted each year. And yet, even after throwing that much salt at the process, obtaining precise percentages in each finished loaf seems a moving target.

It is our purpose today to review the known techniques for salting mozzarella and to evaluate their effectiveness. Each technique will be analyzed, considering its contribution to total productivity. Our definition of productivity is a simple one. We say the most productive procedure is that which makes best use of its resources - in plant, capital, and labor. As each alternative is evaluated, one needs to place at least an approximate mental cost on the plant space used, energy consumed, capital investment required, maintenance costs, labor, etc. In addition, each technique needs to be thoroughly scrutinized for its impact on the environment. As public awareness grows, waste disposal methods that are almost acceptable today may very well be dealt with harshly tomorrow. The truth is, many plants that are guilty of improper disposal have just received, or are about to receive - a wake up call!

Salting Alternatives - The Techniques

Let's take a look at the tables we have prepared and compare the various techniques used to salt mozzarella (see pages 8 thru 11). Then we'll estimate how these approaches challenge the environment, their relative productivity, and the quality of the resultant product.

Before the Cooker

When salting occurs before cooking and stretching, it is applied manually, semiautomatically, or automatically, using pumps, conveyors, augers or various pneumatic techniques.

In the Cooker

The cooker is manually salted by adding prescribed weights (or volumes) of salt at various, predetermined intervals. Other methods include pumps to continuously add salt concentrates, and various devices to fold in dry salt or salt slurries.

After the Cooker

There is a mechanical system that adds salt after the cooker through the use of an auger equipped salt "mixer." A constant stream of salt is added to the flow of mozza from the cooker and the salt/mozza extrusion is auger mixed before molding into loafs.

Before Molding

Salt injection experiments have been conducted to inject a salt concentrate solution into mozza as it is being molded. Measured amounts of salt concentrate are timed so they are added to each mozza loaf as it is being molded.

Before Brining

Before brining, the recent innovation of salt infusion adds precise amounts of salt to each mozza loaf. In partial salt infusion, the process itself adds .5% to 1.0% salt. The balance is provided during convention brining.

In the Brine

Conventional brining salts and cools the cheese.

Brineless

Brineless mozzarella cheese making is now possible, using the salt infusion process to add the necessary salt. Systems now being considered will package the molded and salted loafs and then cool using chilled air or a variety of other, environmentally safe cooling media.

The Salting Alternatives - How Productive are They?

The productivity of a particular process is variable and contingent upon the care and feeding of the system by the processor. Certainly we are all aware of identical processes yielding very different results from the standpoint of total yield and product quality. Nonetheless, certain inherent differences in salting generally promise quite different results, no matter how carefully one adheres to good practices.

The Basic Challenge

Throughout the food processing industry, blending ingredients is a rather exact science. Engineered systems to monitor flow, add ingredients, mix, blend, and etc., have advance steadily over years. With access to all of this technology, one would think that adding salt to mozzarella cheese would be an easy task. It isn't.

In a nutshell, the problem is being able to continuously and accurately monitor the flow of the mozza. Salt is a fairly simple enough material to control but the very nature of mozza sets up barriers. It's fibrous, elastic, and varying density combine to form a target process engineers have been unable to hit consistently. Flow meters capable of peeking inside an extrusion of mozza haven't been design yet. The result is, when automation devices designed to add salt to mozza are analyzed,

we witness a fairly steady volume of salt being added to an almost continuously varying volume of mozza.

No Pre-Salting

While many cheese makers believe it is absolutely necessary to salt at the curd stage, others donot pre-salt. Starter suppliers such as Rhone Poulenc have told us balanced systems can be achieved where pre-salting is not required. With a balanced system, only the hazards from brining have to be dealt with. Of course, when there is sufficient volume to justify ultra filtration to keep the brine properly conditioned, there is an ongoing maintenance cost but minimal impact on the environment. Conventional brining is a slow process, offering excellent control, but it takes up large amounts of floor space and is challenged by disposal problems

Pre-Salting before Cooking

Manual salt applications are approximate because distribution is a rather "average" process. Automatic salt application systems are only as dependent as their ability to anticipate the flow rate of the mozza cheese curd, which is a decidedly difficult task for such a variable process. The result is more or less salt at the beginning and end of a vat with cyclical errors within each run.

In addition, systems using pneumatics for salt delivery may be subject to early equipment failure. Airborne salt has been found to be particularly destructive to weldments. In plants where pressurized positive or negative air flow is employed to distribute salt, salt particulate "dust" can be found attacking electrical wiring terminations, motors, controls, instruments, etc. Air delivery systems that pull or drive salt can also easily fracture their delicate crystal structure, negatively impacting their absorption characteristics.

Finally, whenever pre-salting is employed, salty whey is expelled and a waste hazard is produced. Because this process also includes brining, additional resources and costs are involved. While pre-salting reduces total brining time and the throughput is accelerated, today's environmental pressures may be causing the savings/cost curves to converge.

Pre-Salting and Salting in the Cooker

To add as much salt as possible up front, sometimes pre-salting is accompanied by adding salt in the cooker as well. Productivity does increase as maximum amounts of salt are maintained throughout the process but at what price? While the throughput is enhanced, the losses from salty whey are considerable. The process is high speed but generates considerable waste.

Mechanical Salter

This salter employs a special mixer after the cooker-stretcher and before the molder to add a continuous stream of salt. Again, maintaining accurate flow control is all but impossible with mozzarella. The result finds approximate "dosages" of salt delivered, producing loafs that can be salted heavier or lighter, depending upon the whims of the mozza. In addition, salt added in this manner isn't completely mixed so concentrated "clumps" of salt are present along with frequently heavy coats of salt on the outside of the loaf after molding. The result can be very salty loafs and contamination of the sweet cooling water in the molder. The many problems inherent with mechanical salt mixing accounts for its lack of success in the market. This process is high speed but it is highly inaccurate and very definitely creates waste disposal problems.

Salt Injection Experiments

Experiments have been conducted to inject salt concentrate into the mozza loaf while it is beingg molded. Unfortunately, the process badly tears and rips open the outer skin of the loafs, creating holes, fissures, pockets, and etc. Very little immediate absorption takes place, often causing the solution to pour right back out from the cavernous holes caused by injection. Oozing salt concentrate also contaminates the sweet water used for cooling in the molder. If it is successful in the future, salt injection would be suitable for high speed mozza processing. Its current inaccuracy and l contamination faults leave much to be desired.

Partial Salt Infusion

In partial salt infusion, salt is infused or added before brining and the balance added in the brinne where cooling also takes place. Salt infusion is an accurate and closed process, so it takes place without producing waste hazards. Partial salt infusion offers excellent productivity increases in higgh speed operations but is burdened by the hazards brought from the final brining.

Complete Salt Infusion

With a balanced make procedure in place, the total amount of salt necessary for a particular recipe can be accurately added by salt infusion. It can be said this technique offers cheese makers the best salt control available top them at this time. With the elimination of salty whey products anod the hazards of a brine system, and with the use of environmentally safe cooling systems, maximum efficiency should result. The productivity of this system appears to surpass all other techniques.

Salting Alternatives and Mozzarella Productivity

Basic Concepts, Inc.

How Productive Are They?

	Salt is Added -						
	Before Cooker	In the Cooker	After Cooker	Before Molding	Before Brining	In the Brine	Brineless
Techniques Employed	Manual Automatic Mechanical Pneumatic	Manually added Continuously added salt or salt concentrate	Mechanical feeder adds salt and mixes it into mozza	Injector system injects salt concentration into mozza loaf	Automatic Infusion system adds up to 2.0% of salt	Open Channel Serpentine Pit	Individual Loaf salting with environmentally safe cooling
No Pre-Salting	Balanced System					Total Salting in Brine	
Environmental Challenges						Cleaning/ Disposal Problems	
Productivity						Slowest Procedure	
Quality						Good Quality	
Presalting Before Cooking	To control Ph					Balance salt Brine	
Environmental Challenges	Some Salty Whey					Cleaning/ Disposal Problems	
Productivity	Slows with Salt Increases					Slow Procedure	
Quality	Less Variable					Some Variances	

Salting Alternatives and Mozzarella Productivity

Basic Concepts, Inc.

How Productive Are They?

	Salt is Added -						
	Before Cooker	In the Cooker	After Cooker	Before Molding	Before Brining	In the Brine	Brineless
Pre-Salting Before/While Cooking	Variable 1%	Till Cheese Floats				Balance Reqd.	
Environmental Challenges	Salty, Fatty, Whey	Salty, Fatty, Whey				Cleaning/ Disposal Problems	
Productivity	Slows with Increases	Slows with Increases				High Speed Procedure	
Quality	Wide Variance	Wide Variance				Wide Variances	
Mechanical Salter	To Control Ph		Up to 1%			Balance Reqd.	
Environmental Challenges	Salty Whey		Salty Whey, Salty Coolant			Cleaning/ Disposal Problems	
Productivity	Slows with Salt Increases		Slows with Increases			High Speed Procedure	
Quality	Less Variable		Wide Variances			Wide Variances	

Salting Alternatives and Mozzarella Productivity

Basic Concepts, Inc.

How Productive Are They?

	Salt is Added -						
	Before Cooker	**In the Cooker**	**After Cooker**	**Before Molding**	**Before Brining**	**In the Brine**	**Brineless**
Salt Injection Experiments	Up to 1%			Up to .5%		Balanced Required	
Environmental Challenges	Salty Whey			Salty Coolant (losses)		Cleaning/ Disposal Problems	
Productivity	Slows with Increases			Does not effect speed		High Speed Procedure	
Quality	Wide Variances			Process permanently rips skin		Wide Variances	
Partial Salt Infusion	None- Balanced System				.5% (typical)	Balance Reqd. Very cold brine	
Environmental Challenges					None	Cleaning/ Disposal Problems	
Productivity					Improved Throughput	Improved Throughput	
Quality					None - Deli Quality	None - Deli Quality	

Salting Alternatives and Mozzarella Productivity

Basic Concepts, Inc.

How Productive Are They?

	Salt is Added -						
	Before Cooker	In the Cooker	After Cooker	Before Molding	Before Brining	In the Brine	Brineless
Complete Salt Infusion	None- Balanced System						Up to 2.0%
Environmental Challenges							None
Productivity							Most Productive Technique
Quality							None - Deli Quality

Suummary

With mozzarella, competitive markets and profitability have been the driving forces that have detetermined how the cheese will be processes, including how salting will take place. Ever constant maarket pressures have caused the producers to continuously search for techniques that will make it posossible to significantly increase throughput. The total cost of salt, and faster, more reliable methods to a add salt are more often being seen as additional limiting factors in increasing productivity without conconsiderable capital investment.

It also seems inevitable that the advantages of pre-salting will soon be outweighed by theieprohibitions from local municipal sewer commissions and the various Departments of Natural Reiesources. Chloride restrictions may very well become the tail that wags the dog.

With balanced cheese making and pre-salting eliminated, new automatic salting systems offer exoccellent promise in reliably salting mozzarella and significantly improving throughput while restriricting contaminants to final brining. Results of field production tests seem very heartening to datate.

Finally, there seems little doubt brineless mozzarella cheese making is simply around the corrner. There is no time like the present to start investigating the possibilities . . . before your competetitors beat you to the punch.

Thhank you

I was privileged to have the opportunity to discuss salting alternatives with many cheese makers acrrross the country. Their input has made it possible for our engineering teams to make siggnificantadvances in improving automatic salting techniques. Thanks to each of you for your help.

Thanks also to other industry contributors other individuals who graciously gave me their time, incccluding Dave Strietelmeier, Technical Director of Morton Salt, Dr. Bill Wendorff, University of Wi/isconsin, Madison, Dr. Dave Barbano, Cornell University, and Bill Knoespful and Norm Woodof Rhhone Poulenc.

**Special Thanks to
the Wisconsin
Milk Marketing Board
for their support and
donation to our Open House.**

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