PINK DISCOLORATION OF MOZZARELLA CHEESE

By

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ABSTRACT

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The present study was conducted for a dairy manufacturer wishing to find out what was causing pink discoloration in some of their shredded cheese. The probable causes of the defect such as ingredients, packaging, storage temperatures, contaminating bacteria, and starter cultures were researched. It was determined that the root of the problem probably lay in the oxidative metabolism of certain starter cultures. A rapid screening test, which was developed in the 1960's by University of Wisconsin - Madison, was used to determine the tendency of currently used culture strains to produce a color reaction in the test media.

Thirty-seven mixed cultures of high-temperature *streptococci* and *lactobacilli* from 7 plants were studied to determine if the rod or cocci starter culture could be the cause of pink discoloration. When all tests were completed, 2 of the 7 plants had shown positive

results. These results were not consistent in all trials. This questions the validity of using the Rapid Screening Test to select cultures for use in the production of mozzarella cheese.

Ten single strain cultures found in the mixed combinations, which had caused positive results were purchased for further testing. Three rod cultures from a plant producing negative results in Phase I testing were used. A control test was done using no cultures. All of the cultures showed varied results. All but two had at least one positive trial.

The results of phase II testing were not significant, but do suggest that there is the possibility that all rod cultures may have the characteristic defect to some degree. It is possible that the results of the test may be an indicator to the degree of severity of the defect, however, this was not proven in this project. Another possibility is that the higher the rod/coccus ratio, the higher the chance that the discoloration will occur.

As the shelf life of Mozzarella cheese continues to lengthen through scientific advances, pink discoloration may become an economic problem for cheese manufacturers. Starter culture manufacturers will need to provide their customers with product that will minimize this defect.

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Introduction

General Introduction of the Mozzarella Cheese Industry

In the past decade Mozzarella has become a favorite cheese of retailers as well as consumers. Mozzarella cheese is an elastic and supple product that can be sliced quite easily. When it is cooked, Mozzarella becomes extremely stringy and is the cheese of choice for pizzas (Ridgway, 1999). Pizza industry sales were expected to rise 4% in 2002 and by 25% in 2005. Pizza restaurant growth continues to outpace overall restaurant growth in the United States (Foremost Farms, 2001). The shredded form of Mozzarella is especially popular in all sectors because it is ready to use.

Originally, mozzarella cheese was produced in southern Italy from buffalo's milk. Today, the United States is the leading manufacturer (Early, 1998). Total production of Italian cheeses in the United States in 2000 was 259 billion pounds. Mozzarella made up 210 billion pounds of that total (National Agricultural Statistics Survey, 2000). Historically, American style cheeses have been the number one product of cheese plants in the United States. Lately, mozzarella production has been steadily increasing and American cheese production has been on the decline (Early, 1998). In 2000, 287 billion pounds of American cheese were produced (National Agricultural Statistics Survey, 2000). In the Midwest United States, cooperatives are producing more Italian style cheese than American cheese. In 2000, one of the top ten US cooperatives manufactured 211.3 million pounds of American cheese and 226.5 million pounds of Italian cheese. Then in 2001, they again saw an increase in Italian cheese to 251.2 million pounds but American cheese

the trend nationwide as consumers are eating on the go more often.

Mozzarella production.

In the United States, Mozzarella is made by first standardizing and then pasteurizing raw whole, low fat, or nonfat milk. Because the composition of cow's milk varies due to seasonal changes in feed and by breed, the milk must be adjusted to an appropriate butterfat and solids level before the production begins. A popular way to do this standardization is with added nonfat dry milk (NFDM). The expense of separation can be minimized and the fortification will increase calcium content and decrease moisture content. The resulting cheese has a firmer texture which works well for shredding (Yun et al, 1998).

Another type of milk that is currently being used because of surpluses on the west coast is Ultrafiltration (UF) milk. This milk is concentrated three times before shipping to the Midwest for use in cheese production. Experiments are being done on reducing the lactose level in this milk. Lactose causes cheese to brown as it is cooked. Reducing the lactose in the cheese milk will result in a whiter cheese upon baking (Foremost Farms, 2002).

The most common method of pasteurization is a continuous method. This provides a substantial time and energy savings over the batch method. High temperature short time (HTST) pasteurizers utilize plate heat exchangers. The heating medium is either hot water or steam. In order to destroy pathogens and the spoilage enzyme phosphatase the milk needs to be heated to 72°C for not less than 16 seconds. The milk is then tested for alkaline phosphatase, if negative; it is deemed pasteurized (University of Guelph Ontario, 2002).

After pasteurization the milk is warmed to approximately 100°F and lactic acid-producing bacterial starter cultures are added. The cultures convert lactose to lactic acid. During coagulation the lactic acid is important for firmness and ultimately for cheese yield.

The rate at which the acid develops affects the colloidal calcium phosphate, which has an effect on the rheological properties. Conditions must be controlled or the acid production

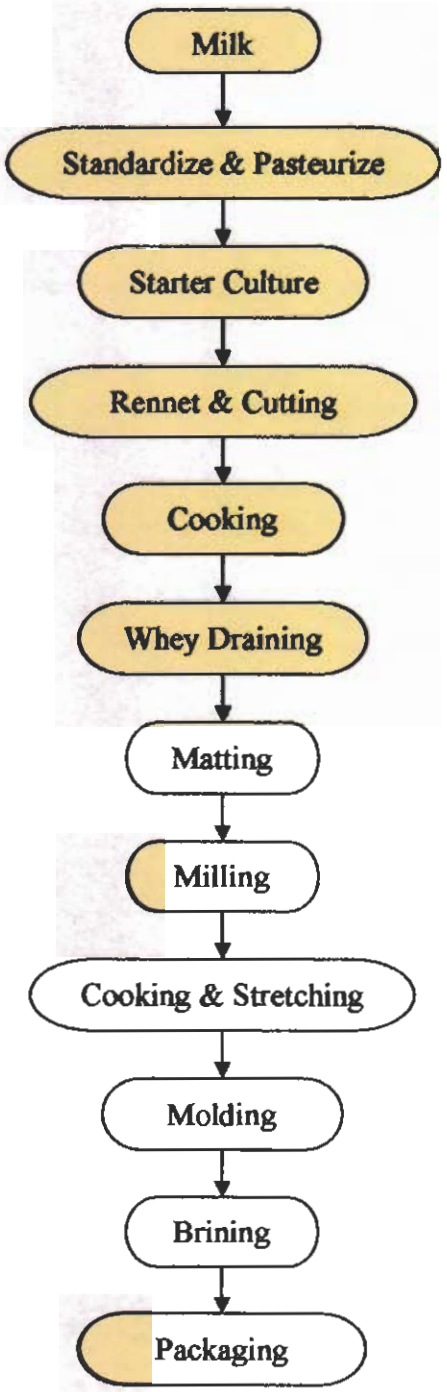

Figure 1: Current Mozzarella manufacturing process as viewed by the author in a number of Midwest plants.

will be too slow and too much moisture will be lost. Lactic acid also plays an important role in the flavor development of the cheese (Oberg, 1994).

Most mozzarella cheese makers use a combination of rods and cocci starter cultures. Common strains include *Lactobacillus delbruekii ssp. Bulgaricus* or *Lactobacillus helveticus*, which are rod shaped bacteria and *Streptococcus thermophilus* which is a cocci (Yun et al, 1995).

The rod to coccus ratio is important because of its effect on proteolytic activity. Cocci do the majority of the fermentation during the first part of cheese processing. A popular cocci, *S. thermophilus* metabolizes the glucose portion of lactose. The rod cultures take over toward the end. A higher number of viable rods will result in deeper and faster proteolysis. The residual galactose is released into the medium. Cheese manufacturers tend to prefer the rod culture *L. helveticus* for mozzarella because the strain will ferment galactose. Galactose is not desired because it causes a Maillard reaction in the cheese upon cooking. Pizza consumers prefer not to have their cheese brown (Foremost Farms, 2002). *L. bulgaricus*, another rod culture, ferments both glucose and galactose. Researchers are finding that the amount of inoculation has a more profound impact on the properties of the cheese than the rod to coccus ratio (Yun et al, 1995).

Rennet is added to coagulate the milk and form curd. A small portion of the rennet remains in the cheese and plays a role in flavor and texture development. In the production of mozzarella, care must be taken to choose a coagulant that is able to withstand the high temperatures that occur during the cooking and stretching stages. The type of coagulant will affect the extent of proteolysis during storage. Commonly used

coagulants include arechymosin, microbial rennet and pepsin acquired from bovine or porcine (Oberg et al, 1994).

The curd should be monitored as it becomes increasingly acidic. At the proper time the curds are cut and then scalded. The scalding at approximately 175°F leads to a low moisture cheese with firm body (Varnam and Sutherland, 1994).

Whey draining, matting, and milling often occur in the same piece of automated equipment. The curds travel on a mesh belt that allows the whey to drain off, matting occurs and the mat is milled into small cubes. The hot curds are forced into an extruder that combines them into a solid mass and provides the necessary stretching characteristic of mozzarella cheese.

After stretching, the cheese is molded into blocks, cooled and placed in cold brine for up to 12 hours. Salt is important for physico-chemical changes that occur during aging. It exchanges with calcium, which is necessary for the emulsification of fat within the protein matrix of the cheese. If the salt content is too high, unfermented sugars will be left in the cheese and browning will occur when cooked at high temperatures (Kosikowski and Mistry, 1997).

Finally, the blocks are either processed for retail sales or packaged for further processing by a wholesaler.

A broad variety of new value-added cheese products are available to the consumer. As these products are being developed and distributed there is a demand for unique physical characteristics desired by the consumer, a cheese manufacturer must have a good understanding of the physico-chemical properties of cheese constituents at the

molecular level. This is important for the control and elimination of any defects that may surface.

Statement of the Problem

In 1999 a cheese manufacturer was notified of a problem that had begun to surface and had not been seen in Mozzarella cheese before. Some of the shredded samples that wholesalers had held for 6 months were turning pink. Before the bags were opened they appeared off-white to pink. Immediately after opening the bag, the shreds turned from pink back to a normal color for mozzarella cheese. At this time the cheese had passed the code date. Soon after noticing the color change it was determined that some samples were turning pink as early as 3 months. The color was distributed uniformly throughout the shred. The cheese did not have a yeasty odor nor were the packages blown up. Once the package was opened oxygen had access to the product causing the color change. Since that time, the pink discoloration has also been found in blocks of mozzarella cheese. The cheese industry is very secretive but a wholesaler has claimed that shreds from a number of manufacturers are exhibiting this problem.

Objective

The purpose of this study was to determine if the rod or cocci starter culture could be the cause of pink discoloration in mozzarella cheese produced by one manufacturer.

Chapter Two

Literature Review

Cheese Discoloration

For many years research has been done on the causes of pink discoloration in cheeses. However, the focus has been on Swiss cheese, annatto-colored cheeses (Hong, Wendorff, & Bradley, Jr. 1995) and Italian cheeses other than Mozzarella (Shannon, Olson, & von Elbe. 1966, 1968). Currently there are very few research documents available on the discoloration of Mozzarella cheese. The cheese industry is very competitive and does not share research done through company research laboratories (Foremost Farms, 2002).

Swiss Cheese Defects

In a study done by Iowa State University (Park, Reinbold, & Hammond, 1967), the development of a pink discoloration 1 to 2 cm under the surface of Swiss cheese was influenced by the strain of propionibacterium used. *P. shermanii* always exhibited the defect, whereas *P. arabinosum* showed the pink coloration less often. This discoloration occurred during the final stages of cooling while being cured at 2.8 to 7.2°C. In both cases, the color faded within a few hours after the cheese was cut and packaged. It was discovered that the pink zone was related to oxygen diffusion into the cheese. The temperature of the "warm room", where the eyes of Swiss cheese develop, did not have an significant effect on the percentage of cheese that developed the pink zone (Park et al, 1967). This problem was alleviated before reaching the consumer.

Annatto-Colored Cheese Defects

Since the 1930's pink discoloration in processed annatto-colored cheeses have been researched. This defect was and still is seen at the time of manufacturing. To prevent economic loss, the product is reworked into other products (Shumaker and Wendorff, 1998). The type of colorant, process temperature, use of emulsifying salts, colored cheese usage, age of the cheese, pH of the cheese, and the use of additional whey solids were studied by Shumaker and Wendorff (1998), as possible contributors to the pinking of processed cheese. They found that when aqueous based annatto colorants were added, the defect occurred. Increased processing temperatures contributed to the color change (Zehren and Nusbaum, 1992; Shumaker and Wendorff, 1998). Cheeses made with sodium citrate showed the color change more often than disodium phosphate. Cheese colored with norbixin, even in small amounts, caused pink discoloration (Zehren and Nusbaum, 1992). The same study also determined that the use of aged and acid cheese to produce process cheese contributed to the pink discoloration. Hong et al. (1995) noticed pH played a role in the defect. Pinking occurred when the pH of the cheese was reduced from 5.4 to 4.8. When acid whey solids were used in the production of processed cheese the cheese exhibited the defect. Sweet whey showed some resistance to the problem.

Cooking temperatures seemed to play an important role in the color change. The cheese food was more susceptible to pink or brown discoloration when produced with higher cooking temperatures. The color occurred less quickly if the cheese was cooled immediately after production (Kosikowski, 1982).

High-intensity lighting in grocery stores catalyzed the pinking effect in annatto-colored cheeses. Cheeses were tested under 250 foot-candles and it was determined that pinking occurred in as little as two days. Temperature abuse of the retail packages in the grocery stores

has long been known to cause spoilage and enhance the rate of the defect development (Hong et al, 1995).

Italian Cheese Defects

(Shannon et al.,1966) studied Pink discoloration in Italian varieties of cheeses. They examined Romano and Parmesan varieties. They found uniform bands of discoloration beneath the surface, irregular bands following cracks or uniform discoloration throughout cheese. The pink band started at approximately 2.5 cm below the surface and spread throughout the entire cheese. It was usually pink but sometimes became orange-brown in older cheese. The discoloration was also observed in Provolone, Asiago, and Fontina cheeses. The defect was not related to flavor quality. The pink color faded when exposed to air but was stable when maintained in nitrogen (Shannon et al., 1966).

Currently, no research on the pink discoloration of mozzarella cheese can be found. However, all of the Italian cheese defect findings as stated above align with the problem that is being seen in Mozzarella cheese.

Possible causes of Pink Discoloration in Italian Cheeses

The discoloration of the Italian cheeses was associated with the nondialyzable fat-free fraction. Fat from the normal and discolored cheese was the same. There wasn't any difference in the dialyzable materials. The color was found to be covalently bounded to the protein or was the water-insoluble portion of the casein, which has been changed through oxidation. No relationship between discoloration and pH, sensory qualities, levels of fat, moisture, NaCl, and soluble nitrogen were found (Shannon et al., 1966).

A survey of cheese manufacturers (Shannon et al., 1968) showed that they did not feel that the defect was caused by the use of bleaching agents and lipolytic enzymes, seasonal effects, or any particular variety of hard cheese.

Effects of Modified Atmosphere Packaging

Shredded mozzarella cheese is becoming more and more popular in the North American market. Because of the increased surface area available to spoilage organisms, modified atmosphere packaging has been studied (Eliot et al., 1998). It was determined that CO_2 levels of $\geq$ 75% were most appropriate for maintaining microbiological quality and safety of shredded Mozzarella cheese. This has become the common guideline for the packaging of shredded cheeses. Because of the decreased level of oxygen in the packaging, it is possible that if pink discoloration occurs, it would remain apparent to the consumer until the package is opened. (Shannon et al., 1966) determined that the intensity of the pink color, of Italian cheeses other than mozzarella, was held constant while under nitrogen. Once exposed to air, the pink color disappeared. Therefore packaging does not cause the discoloration but it can accentuate the problem if it is present.

The packaging system at one of the plants in this study was observed. The O_2 and CO_2, and nitrogen flushing are checked every ½ hour. The gas content of newly packaged shredded cheese was the same in every bag that was packaged. The gas content of packages of shredded cheese with the defect was found to be the same as other packages that did not show the defect. The product was not stored before being packaged. All cheese was shredded and packaged at the same temperature.

Storage Temperatures

Storage temperatures played a vital role in the development of the discoloration. Research shows that discoloration of the cheese can be minimized by using lower storage temperatures, 4°C as compared to 15.5°C (Shannon et al., 1969). This again would amplify the problem but would not be a cause.

Storage temperatures in the plants involved in the study were all the same. The time from packaging to proper storage temperature was found to be consistent.

Psychotropic organisms

Psychotropic organisms can also produce pink discoloration in cheese; however, the cheese has a mottled appearance. The bacteria are not all killed in the pasteurization process and live in pockets in the cheese (Hicks, 1982).

Ingredients

A possible cause for the discoloration defect in the ingredients was researched among the plants involved in the study. It was determined that all plants used common ingredients but only three plants produced cheese with the discoloration. Of the cheese produced in those three plants, only a small portion was found to show pink discoloration. All of the plants in the study use the same supplier for their non-fat dry milk. Raw milk was procured from many farmers throughout the Midwest but all milk met quality standards that are the same at all plants. Blend A, a unique ingredient formulated by the processor was used in both cheeses with the defect and those without. Ultra Filtrated milk, a 3x condensed product, is purchased and used in a number of the plants. Italian concentrate is another product that is produced and used by the processor in all plants. Whey Protein Concentrate is used all plants.

Brine temperature and holding time was consistent among batches of cheese known to cause the defect and those that didn't. Salt and pH of the finished product was recorded as being similar in all of the cheese produced in the plants.

Starter Cultures

Because pink discoloration in mozzarella cheese has a uniform development, a homogeneous distribution of the agent of defect throughout the curd is suggested. Research shows that lactic starter cultures are uniformly distributed throughout the cheese (Dawson and Feagan, 1957; Dean, Berridge, &. Mabbitt. 1959).

During a 2-year study (Shannon and Nelson 1969) only defect-associated strains of starter culture were detected as causing the discoloration. None of the strains were cocci; all of the strains were rods. *Lactobacillus Helveticas* caused the most intense color change, turning the cheese pink. *L. burglaricus* also caused pink coloring in the cheese. Cheese made with *L. lactis* did not develop the defect. When strains that caused the discoloration were mixed with strains that did not cause the discoloration there was no change in the intensity of the color. Using *Streptococcus thermophilus* delayed the defect but didn't prevent it.

Oxidative Metabolism of Italian Starter Cultures.

All types of organisms can adjust the oxidation-reduction potential of their medium to a definite zone, which will usually change during the successive phases of growth, and that growth can only occur between certain limits (Davis, 1934).

Bacteria control oxidative reactions by removing molecular oxygen through their respiratory processes and also by producing a system capable of inducing a high reducing intensity in the medium through fermentative reactions (David, 1934).

Individual strains of lactobacillus differed in their effect on the reduction-oxidation potential of cheese. In a study (Shannon and Nelson, 1969) strains that were known to cause

the defect always created more highly oxidized conditions within 48 hours after the cheese was made. Then within 8 weeks the reduction-oxidation potential changes and the entire cheese is reduced except the surface. Strains that were not found to cause the discoloration produced reduced conditions within the first 48 hours after the cheese was made. They also completely reversed their reduction-oxidation potential and were more highly oxidized at the end of an 8 week trial (Shannon et al, 1969).

It is possible that the oxidative metabolism of certain strains of lactobacillus starter culture play a significant role in the development of the pink discoloration of mozzarella cheese.

Rapid Screening Test

In an effort to determine the tendency of starter cultures to produce pink discoloration, a rapid screening test was developed (Shannon and Nelson 1969). There is no literature to support any rapid testing done on starters used specifically to produce Mozzarella cheese. Calcium carbonate in a skim milk medium was used in the rapid screening test. Organisms causing the defect in the cheese turned the medium brown in the test. Those organisms not exhibiting the defect in cheese always had no color reaction. A phosphate system was also developed but the results were not as discernible as in the calcium carbonate system. When test tubes were used, results were erratic. Prescription bottles gave good results (Shannon and Nelson1969).

This test was developed in 1969 and there is no evidence in literature that another rapid screening test has been developed for detecting which strains of starter culture may contribute to the problem of pink discoloration.

The present field study was conducted for a dairy cooperative wishing to find out what was causing the defect in some of their shredded cheese. The probable causes of the defect such as packaging, storage temperatures, contaminating bacteria, and starter cultures were

researched. It was determined that the root of the problem probably lay in the oxidative metabolism of certain starter cultures. The test was performed to determine if current strains of starter culture being used by the cooperative show a positive result thus indicating that the culture was probably the problem.

Chapter Three

Materials & Methods

Materials

All starter culture combinations used in the production of cheese made at 7 mozzarella plants were tested in the study. Coccus culture combinations were used as controls in phase I. These strains had been proven in previous studies not to turn the screening medium brown (Shannon et al., 1968). During phase II the tests were performed with single strains of rod and coccus obtained from Chr. Hansen, Inc., Milwaukee, WI; Rhodia, Inc., Madison, WI and Degussa BioActives LLC, Waukesha, WI. A control was formulated without the use of starter bacteria in phase II. Non-fat dry milk powder, whey powder, and Italian concentrate powder were provided by a local Dairy Cooperative. Calcium carbonate was purchased from VWR Westchester, PA.

The Italian Concentrate Media that was used in this study is produced by a Midwest dairy processor and contains whey, yeast, diammonium phosphate, monosodium phosphate, magnesium sulfate, manganese sulfate, and zinc sulfate. This whey based medium provided a high quality starter with optimum activity and phage protection.

Equipment

An autoclave was used to sterilize equipment, containers, and solutions. This was done at 121°C with 15 – 20 PSI/unit of steam pressure. The following sterile glass containers were used: (13) 300 mL Reagent bottles, (40) 150 mL dilution bottles, and (4) 2000 mL Erlenmeyer flasks. A scale accurate to three decimal places was used to weight the non-fat dry milk powder, whey powder, Italian concentrate powder and calcium carbonate. A Corning Stirrer/Hotplate model PC620 (VWR International, Westchester, PA) was used to mix the phase I and II test culture medias. Two 100 mL sterile graduated cylinders and sterile pipettes,

1 mL and 5 mL were used for transferring fluids. A water bath with oscillator (VWR International, Westchester, PA) was used in phase II to sterilize the culture media and then hold the inoculated media in suspension at 43°C until the proper pH was derived. A pH meter (VWR International, Westchester, PA) was used to measure the pH of the inoculated media. An incubator (Chicago Regional Distribution Center, Batavia, IL) set at 37°C was used to hold the cultures for a 10 day observation period.

A spectrophotometer (VWR International, Westchester, PA) was used to try to establish a value for the color of the test results. However, the machine did not detect any color absorption in the visible spectrum of either the positive or negative samples.

Methodology

Phase I Testing

Mixed Cultures. The starter combinations were prepared at the plants for normal cheese makes. A 30 mL sample was taken just before use in cheese making and frozen immediately at -80°C. After completely frozen, the sample vials were shipped next day mail to the University of Wisconsin – Stout. Upon arrival they were immediately placed in the refrigerator. Samples were inoculated into the test media within two days of arrival.

Preparation of Calcium carbonate – skim milk medium. Non-fat dry milk was reconstituted to an 11% solution. The 11% solution was distributed among 2-liter Erlenmeyer flasks being careful not to fill them more than half full. Aluminum foil was used as a cap for the flasks. They were then autoclaved at 121° C for 15 minutes. Dilution bottles containing 20 g of dry calcium carbonate ($CaCO_3$) were sterilized at 121° C for 15 minutes in the autoclave. The $CaCO_3$ was added as a buffering agent so that the pH of the medium would allow for extended growth of the culture organism. When cool, 100 mL of the sterile milk was aseptically transferred into each dilution bottles containing the $CaCO_3$. Milk samples were then inoculated with test

cultures at the rate of 1% by volume. A total of 37 mixed strains from the 7 cheese plants were used for the inoculation.

The dilution bottles were placed in the incubator and held at 37° C for 10 days. The bottles were shaken daily to redistribute the $CaCO_3$ (Shannon et al, 1968). At 10 days a visual observation of the color change in the media was made and recorded. These colors were Normal (N), Normal yellow (Ny), and Brown (B). The media was a cream color if there was no change in the color of the media. Ny showed a golden yellow coloring of the whey and specified a very dark gold color to brown.

At least three tests were run on each of the starter combinations. These tests were on the same cultures but different make dates.

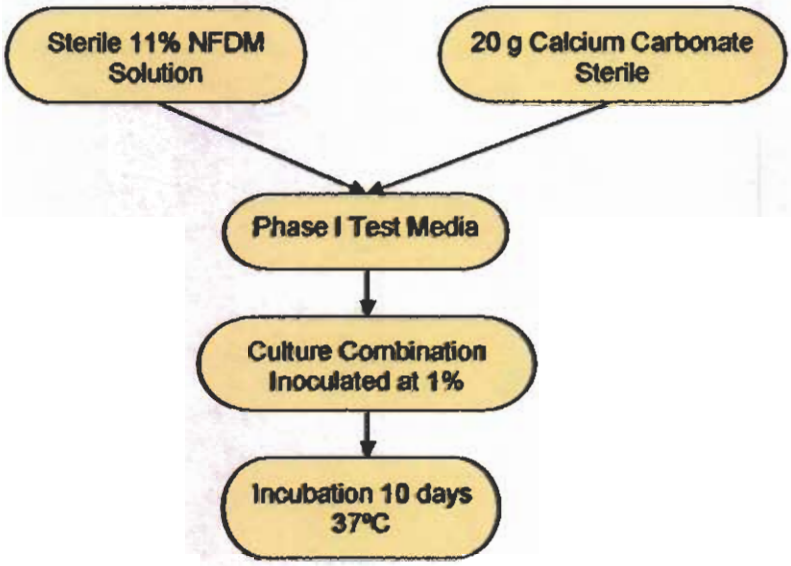

Figure 2: Flowchart of phase I testing.

Phase II Testing

When all tests were completed, ten single strain cultures which had caused positive (B) results were purchased for further testing. Three additional rod cultures that had no history of positive results or discoloration in cheese where also tested.

Culture preparation. Growth media for the starter was formulated by first heating distilled water to 38°C. A known quantity (1.55 L) of the water was then combined with 34 g of Italian Concentrate and 94 g of whey protein concentrate (80%). This recipe was doubled in order to make enough media to grow all cultures properly.

After mixing, 200 mL was poured into each of thirteen labeled reagent bottles. The bottles were pasteurized in a water bath for 1 hour at 88°C. After 1 hour the water bath was cooled to 43°C.

Cultures stored at -80°C were partially thawed in warm water 1 mL of each specific culture was inoculated into a reagent bottle appropriately labeled for that culture.

An initial pH was taken shortly after all cultures had been inoculated. Subsequent recordings were taken until the culture media reached a pH of 5.0 – 5.4.

After reaching the appropriate pH, 3 mL of the culture was ascetically transferred into three dilution bottles, 1 mL per bottle. The dilution bottles had been prepared with the calcium carbonate media as in phase I. This process was repeated for each of the single strain cultures.

The dilution bottles were placed in the incubator and held at 37°C for 10 days. The bottles were shaken daily to redistribute the $CaCO_3$ (Shannon et al, 1968). At 10 days a visual observation of the color change in the media was made and recorded. Colors were recorded as in Phase one. Phase two was repeated so that a second set of observations could be recorded.

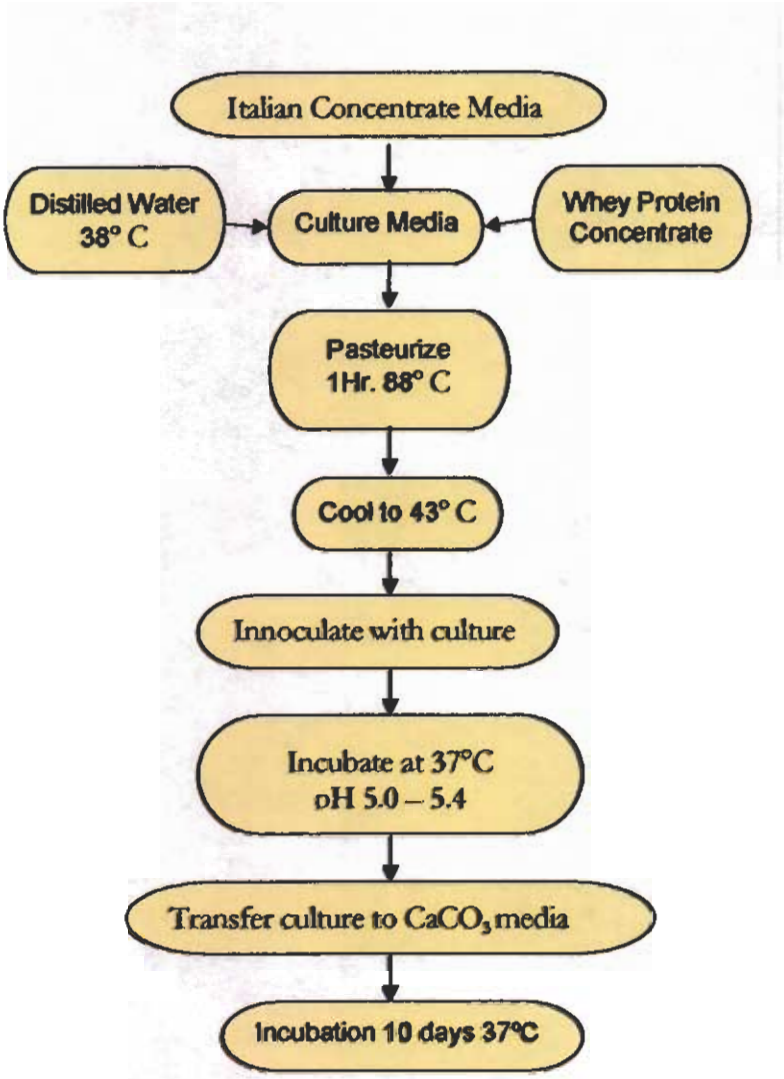

Figure 3: Flowchart of Phase II Testing

Chapter Four

Results and Discussion

Phase I testing results: Mixed Cultures

A total of 37 mixed cultures from seven plants were tested to determine if the rapid screening test could identify combinations that might be causing the defect (Table 1). Only two of the same mixed cultures were used in two of the plants. The other 35 mixed combinations were not repeated in any two plants.

Figure 4. Two questionable (Ny) and five negative (N) test results.

Normal (N) was considered to be negative for the defect causing pink discoloration, slightly yellowed (Ny) was questionable, and Brown (B) was considered positive. The colors were determined subjectively.

Table 1

Relationship between the mixed starter culture and color of screening test medium.

Plant	Mixed Cultures	Culture Species	Screening Test Color Results
1	R110 R160 C390 C410 R110 R160 C257 C370 R110 R160 C310 C360 R110 R160 C370 C390	*L bulgarius S. thermophilus* *L bulgarius S. thermophilus* *L bulgarius S. thermophilus* *L bulgarius S. thermophilus*	N N Ny N
2	C180C280 Rx160R190 C380C430 C90TC257 C390 C420	*S. thermophilus* *L. bulgaricus L. helveticus* *S. thermophilus* *S. thermophilus* *S. thermophilus*	Ny Ny,N N Ny Ny
3	13M14N 3C39S 9125J 32L49G	*L. helveticus S. thermophilus* *L. helveticus S. thermophilus* *L. helveticus S. thermophilus* *L. helveticus S. thermophilus*	N N N N
4	C180C430R170 C257C260R170 C180C400R170 C390C410R170 C330C410 St-4C180C400 C260C430R170 C257 C330 R170	*S. thermophilus L. bulgaricus* *S. thermophilus L. bulgaricus* *S. thermophilus L. bulgaricus* *S. thermophilus L. bulgaricus* *S. thermophilus* *S. thermophilus L. bulgaricus* *S. thermophilus L. bulgaricus* *S. thermophilus L. bulgaricus*	N Ny,N N N Ny Ny N N
5	16L43P 3C39S 5E32X 13M14N	*L. helveticus S. thermophilus* *L. helveticus S. thermophilus* *L. helveticus S. thermophilus* *L. helveticus S. thermophilus*	B,N N N B, Ny, N
6	SC9SC25 HC24HC30 HC25HC29 HC33HC34 HC23 HC27	*S. thermophilus* *S. thermophilus* *S. thermophilus* *S. thermophilus* *S. thermophilus*	N N N N N
7	HC26HC29HR17HR24 HC27HC28HR16HR18 IT11HR16 HC33HC34HR11HR13 Messo (FRRS)	*S. thermophilus L. bulgaricus L. helveticus* *S. thermophilus L. bulgaricus L. helveticus* *L. bulgaricus Cocktail* *S. thermophilus L. bulgaricus L. helveticus* *Cocktail*	B,Ny,N Ny,N B,Ny,N Ny,N N
Control	None		N

As seen in Table 1, the results from multiple trials from all starters collected from plants 1 through 4 and plant 6 were normal or slightly yellowed, indicating negative results.

Two of the mixed cultures that were tested from plant 5 showed positive results by turning brown. In repeated trials both combinations also showed normal and slightly yellowed results. All culture combinations from this plant contained 2 species or rod bacteria and 2 species of cocci. Two of the combinations used in this plant were also used in plant 3. One combination had the same normal results as plant 5 but the other varied significantly.

Plant 7 showed positive, brown results in two of the mixed combinations that they utilize. One contained a *L. bulgaricus* and a cocktail, and the other contained two *S. thermophilus*, a L *bulgaricus* and a *L. helveticus*. A high percentage of the trials turned out slightly yellowed. There were only a few normal results.

A control was produced by following the rapid screening test procedures without the addition of the culture. A negative result was achieved.

The findings were interesting in that discolored cheese had been returned to plants 3, 5, and 7. Plant 3 results were all negative.

Phase II testing results: Single Strain Cultures

Rod cultures from plants 5 and 7 that had shown positive results in phase I were used in phase II testing. Three rod cultures from a plant producing negative results were used. A control test was done using no cultures. A total of 13 strains were grown and tested during phase II. Two tests and a total of six trials were performed with each species of bacteria. The first test was performed immediately after receiving the frozen cultures. The cultures were thawed just enough to supply the required amount for the test. The rest of the culture was returned to the regular freezer and the next test was performed one week later. One culture did not survive the second freezing and no growth was seen.

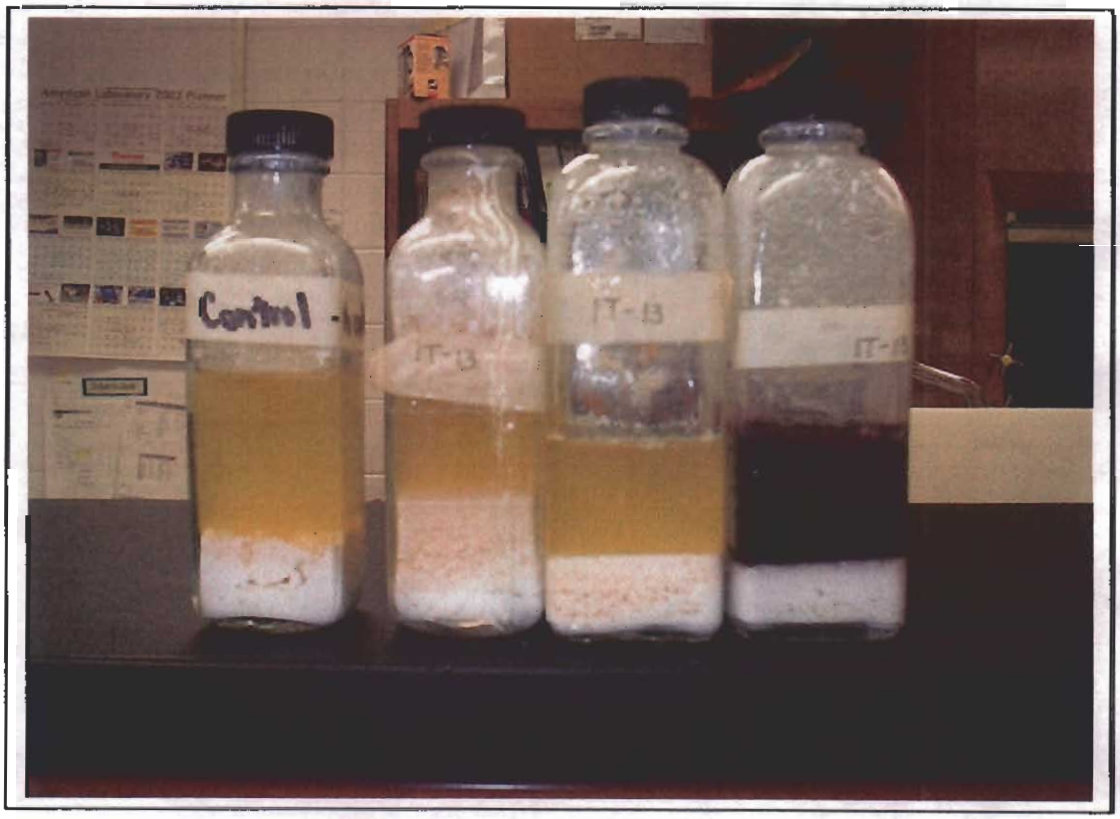

Figure 5. One positive result (B) as compared to the control and two negative (N) test results.

Table 2.

Relationship between the strain of starter culture and color of screening test medium.

Culture	Test1 – Screening test color results			Test2 – Screening test color results		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
M	N	N	Ny	B	N	N
N	N	Ny	N	Ny	N	B
L	Ny	Ny	B	No growth	No growth	No growth
P	N	N	N	Ny	Ny	Ny
R170	N	Ny	B	B	B	B
R160 1754	N	B	B	N	N	Ny
R160 1527	Ny	Ny	B	B	Ny	Ny
17	N	N	Ny	N	N	Ny
24	Ny	B	B	Ny	Ny	B
16	N	N	N	N	B	Ny
18	N	N	Ny	B	B	B
IT-11	N	N	N	N	B	Ny
IT-13	B	B	B	N	N	B
Control	N	N	N	N	N	N

All of the cultures showed varied results. All but two had at least one positive trial. R170 and IT-13 both had 4 positive results. R170 was one of the cultures chosen to test that came from a mixed culture that had not previously produced positive results. In the mixed combination it was one of three cultures, the other two being cocci. Both species of R160 produced two positive trials, and the rest of the trials were yellowed. These rod bacteria also came from mixed culture combinations that had not produced positive results in phase I.

Cultures M, N, L, and P had all previously produced positive results. In phase II M, N, and L each produced one brown trial, P did not. P and L were both in the same mixed culture. It was interesting to note that during the growth of the culture in the second test, these four species all took over 70 hours to reach the proper pH for inoculation. The other cultures that were grown at the same time under the same conditions all reached the proper pH in less than four hours. They must have been weaker cultures that could not stand the second freezing. During the first test they all reached the proper pH in less than 4 hours.

Cultures 17 and 24 were in a mixed combination in phase I, as were cultures 16 and 18. Culture 16 was also in another culture mixture that had a positive result in phase I. Single strain cultures 24 and 18 each had three positive trials, 16 had 1, and 17 had none. IT-11, which the same manufacturer produced, showed one positive trial.

Chapter Five

Conclusions

Interpretations and Conclusions

The results of this study were difficult to analyze because of their subjective nature. The data was not consistent in all trials. This questions the validity of using the Rapid Screening Test to select cultures for use in the production of mozzarella cheese.

Phase I testing showed plants 5 and 7 had positive results. The fact that discolored cheese had been returned to these plants suggests that the test does have the ability to identify mixed cultures that could cause the defect. However, cheese had also been returned to plant 3. Plant 3 used some of the same culture combinations that plant 5 uses. This study resulted in only negative tests at plant 3. So even though the rapid screening test may be able to identify cultures that will cause the discoloration, it may not be with a high percentage of accuracy. Another point to consider is the method of shipment of the samples. The samples received from plant 5 were thawed at the time of arrival, whereas the samples from plant 3 were not.

Another possibility is that the higher the rod/coccus ratio, the higher the chance that the discoloration will occur. This could occur unintentionally if the coccus is knocked out by phage. Currently, treatments of coccus bacteria have produced Isogenic strains that are able to trick phage. This helps to prevent rods from becoming the dominant strain in mixed cultures. The risk of pink discoloration occurring could also increase as more rod cultures are added to a culture combination.

The results of phase II testing were not significant, but do suggest that there is the possibility that all rod cultures may have the characteristic defect to some degree. It could be

that the results of the test could be an indicator to the degree of severity of the defect, however, this is not proven in this project.

Recommendations

As the shelf life of Mozzarella cheese continues to lengthen through scientific advances, pink discoloration may become an economic problem for cheese manufacturers. Starter culture manufacturers will need to provide their customers with product that will minimize this defect. An accurate test for screening cultures should be developed. Research should also be done on the production of mozzarella cheese with all coccus combinations.

As a precaution, the cultures found in phase II that caused the most positive results should be replaced by cultures that had very few positive results.

A shelf life study should be established at the plants where discolored cheese has been produced. This would allow more data to be collected as to which culture combinations may be causing the defect. If none of the cheese was found to be defective 30 days after the normal shelf life of mozzarella, a study of handling of the product after it leaves the plant would need to be done.

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