Microbiological Problems in Dairy Foods in the 1980s

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ABSTRACT

Microorganisms are important to the dairy industry. Some bacteria and molds are used to manufacture dairy products while others cause spoilage or are potential health hazards. Many of the microbiological problems challenging the dairy industry in the 1980s will not be new. Psychrotrophic bacteria will continue to be a problem, but more effort will be directed toward elucidating their effect on processing properties of milk and the significance of enzymes produced by them. Heat-stable enzymes that cause quality problems will become more important as efforts to achieve a longer shelf-life for products are realized. Acceptance of reverse osmosis and ultrafiltration for concentrating milk and whey might increase if energy costs continue to rise or if economic advantages such as increased cheese yield can be accomplished. Microbiological problems associated with the product processed, processing parameters and sanitary design of this equipment will emerge if greater use of the technology is implemented. Microbial production of toxic metabolites and biologically active chemicals such as mycotoxins and amines will emerge as primary factors in the public health area as the research in food toxicology expand.

Microbiological problems of foods can be classified as those associated with (a) spoilage or product quality deterioration, (b) public health and nutrition concerns and (c) food production. Like other foods, microbiological problems associated with milk and milk products can be classified similarly. Major problems associated with an industry do not often change rapidly and are normally associated with a change in technology or new knowledge. Solutions to problems associated with technological developments are a continuous process. Whenever a solution to a particular problem is found, it is discovered that a more subtle problem existed or that the solution implemented caused new problems. It is difficult to anticipate problems. This can be approached from a historical perspective by examining changes that have occurred and the current status of problems generated by that change, or technological changes that might be implemented by the industry can be projected by examining the current state of technological development. By evaluating these anticipated changes, projections concerning potential microbiological problems can be attempted.

PRODUCT QUALITY DETERIORATION

Spoilage and quality deterioration of raw milk and pasteurized fluid products is a problem. Historically, quality deterioration was associated with poor cooling. This resulted in high acid development and off-flavors associated with lactic streptococci and other mesophilic bacteria. The early 1950s, bulk tanks were introduced for farm storage of raw milk. This was a major technological development that permitted rapid cooling and storage of raw milk at the farm. It essentially eliminated milk defects caused by mesophilic bacteria. However, the storage temperatures and time permitted growth of psychrotrophic bacteria. Psychrotrophic bacteria have been and still are a major problem to the dairy industry. These bacteria are the most commonly encountered spoilage organisms in the dairy industry. They grow during extended refrigerated storage and are the result of inadequate sanitation. Several factors exist that will probably cause increased storage times for raw and pasteurized fluid milk products in the future. Milk plants are continually trying to reduce their weekly processing days and increase the shelf-life of their products. Both of these factors will result in increased storage times for milk. Energy costs will make it necessary to consolidate deliveries and transportation of milk. This could result in a reduced frequency of milk deliveries from producers, and any reduction in the frequency of producer deliveries will cause increased storage time for milk at the farm.

Marth and Frazier (34,35,36) clearly identified problems associated with the storage of milk in farm bulk tanks. They found that psychrotrophic bacteria were the predominant bacteria in bulk tank storage milk (34) and that the initial number of bacteria and storage conditions affected the rate of growth and terminal distribution of the microflora in milk (35,36). Other scientists in the United States and Europe have reported similar results concerning the distribution of psychrotrophic bacteria in milk. Randolph et al. (49) found that psychrotrophic bacteria accounted for more than 30% of the total bacteria in individual producer and commingled milk samples in Texas. In a review of available literature, Thomas (60,61) reported similar levels of psychrotrophic bacteria in milk from various European countries. Additionally, he reported that psychrotrophic bacteria were more prevalent in high count than low
count milk. Presence of psychrotrophic bacteria in milk is not as important as the fact that they are capable of rapid growth during refrigerated storage. In 1953, Green and Jezeski (21) reported on the generation times and numbers of bacteria required before proteolysis and lipolysis could be detected chemically. Using two Pseudomonas sp. and Aerobacter aerogenes isolated from creamery water supplies, they reported minimum generation times ranging from 5.43 to 6.52 h. The number of bacteria required before proteolysis or lipolysis could be detected was approximately 10^6 per ml. This indicates that off-flavors will be produced at psychrotrophic counts ranging from 10^6-10^8 cfu/ml. This is also the number of bacteria that are usually found when pasteurized products are no longer acceptable (31).

Recent, and probably future research concerning psychrotrophic bacteria has been directed toward the effects of psychrotrophic growth on the manufacturing properties of milk, heat-stable enzymes produced by the bacteria, and thermoduric psychrophiles. A considerable amount of research has been reported concerning the effect of psychrotrophic growth and milk storage on cheese yield (3,12,13,25,27,44,46,47). Results have been variable, but a decrease in cheese yield was usually associated with psychrotrophic bacterial growth or increased storage time. Law et al. (27) did not find a decrease in Cheddar cheese yield and stated that at the levels of psychrotrophic contamination usually encountered in commercial milk, psychrotrophic growth did not appear to cause a problem with cheese yield. However, milk used in their study was never more than 72 h old. Bacterial counts and cheese yields recently reported for manufacturing milk cheese plants in the United States make this conclusion debatable (25,47).

Psychrotrophic bacteria produce proteolytic and lipolytic enzymes during growth. Some of these enzymes are heat-stable and survive milk processing temperatures. Even low quantities of enzyme activity can cause product quality problems on prolonged storage. There is no direct link to show that the enzymes responsible for this problem were produced by psychrotrophic bacteria but heat-stable proteases are a problem in UHT products regardless of their source. However, heat stable proteases produced by psychrotrophic bacteria have been isolated and implicated with product quality problems. Law et al. (28) isolated a heat stable protease from Pseudomonas fluorescens which gels UHT-sterilized milk. White and Marshall (67) observed a definite reduction in the shelf-life and quality of cottage cheese and Cheddar cheese when the products were made from milk inoculated with P. fluorescens P26 or the heat-stable protease extracted from the bacteria. Many of the proteases identified with psychrotrophic bacteria are incredibly heat-stable. Adams et al. (2) isolated 10 psychrotrophic bacteria from raw milk that produced heat-stable proteases during growth in sterile milk. Proteases from 9 of 10 isolates retained 70% of their original activity after heating at 149 C for 10 sec. Gegg-Geziabher et al. (18) partially purified heat-stable proteases from 6 of 12 Pseudomonas sp. that produced heat-stable proteases. All of the enzymes retained some activity after heating at 121 C for 10 min. An unusual characteristic of a heat-stable protease isolated by Adams et al. (4) is that it can be inactivated at temperatures lower than those used for ultrapasteurization. It was later demonstrated that the enzyme can be inactivated by a combination of 55 C for 40 min followed by UHT sterilization (66). Other heat-stable enzymes produced by psychrotrophic bacteria might also be susceptible to denaturation by this method. This treatment would not result in the most desirable processing sequence but it is certainly better than bitter or gelled products.

The effect of lipolytic enzymes from psychrotrophic bacteria on milk products is not well documented. Published data concerning these enzymes has recently been reviewed by Law (26). Lipases produced by these bacteria appear to retain sufficient activity after pasteurization to produce rancidity in cream and cheese products. Rancidity has been associated with cheese made from both raw and pasteurized milk which contained concentrations of psychrotrophic bacteria greater than 10^8 cfu/ml (29).

Thermoduric psychrotrophic bacteria were first isolated from milk in 1969 by Grosskopf and Harper (22). Further evidence for the presence of these bacteria in raw milk was soon reported. In 1971, Chung and Cannon (11) found psychrotrophic spore-forming bacteria in 83% of the raw milk samples they examined. They grew slowly at 7 C, having a lag phase of 8-14 days and a generation time of 22 - 26 h in the log phase of growth. Shehata and Collins (54) isolated thermoduric psychrotrophs from raw milk in 1971 and identified them as belonging to the genus Bacillus. Further examination revealed that some of the bacteria were capable of growth at 0 C (55). Generation times for three of the isolates at 7.2 C were 5, 5.5 and 7 h. Plate counts at the time of off-flavor detection were between 10^6 and 10^7 cfu/ml. In 1972, Bhadsavle et al. (5) isolated psychrotrophic Clostridium from raw milk. However, these organisms were anaerobic and grew slowly at refrigeration temperature. Heat resistant Bacillus has also been isolated from stored pasteurized milks (15,48,65). The most extensive study of thermoduric psychrotrophic bacteria in raw milk has been completed by Mikolajcik and Simon (43). They heated raw milk at 80 C for 12 min and determined psychrotrophic counts after the milk had been stored for 7, 14 and 28 days at 7 C. They concluded that heat-resistant psychrotrophic bacteria may cause spoilage of heat-treated milk. Boddyelt (6) recently reported that the incidence of spoilage of milk attributed to these bacteria might be greater than once believed.
However, before too much significance for milk spoilage is attributed to these bacteria, it is important to remember that other psychrotrophic bacteria are still a problem that must be eliminated before the shelf-life necessary to make thermoduric psychrotrophs the major problem can be achieved (24).

It is unfortunate that psychrotrophic bacteria have been a major problem to the dairy industry for 25 years and will continue to be a problem in the future. Previous research has provided an understanding of the numbers and types of psychrotrophic bacteria that will affect the shelf-life and quality of milk and milk products. The knowledge to solve, or at least to minimize, this problem is also available. Psychrotrophic bacteria are in milk as a result of contamination and their growth rate increases as the storage temperature increases. Marth and Frazier (34,35,36) showed that if contamination is minimal and storage temperatures are maintained below 3.3 C, psychrotrophic problems will be minimal. Perhaps it would be better if future research were directed toward controlling the growth or enzyme production of these bacteria rather than enumerating them and determining their effect on raw milk and milk products.

FOOD PRODUCTION DEVELOPMENTS

New and previously available but unused methods of handling milk and manufacturing milk products will be adapted or tried on an experimental basis. The acceptance of these developments will be accelerated by the increasing costs of energy and the opportunity to increase product yields. Some of these methods hold promise for both.

Reverse osmosis and ultrafiltration have been used to concentrate milk and whey (14,16,17,37,36). Concentration of whey has been primarily to achieve a lower cost of concentration but modified whey protein concentrates have been made. Milk has been concentrated to manufacture cheese (14,37) and to reduce the cost of concentration before drying (1). Use of ultrafiltration and reverse osmosis offers the potential for greatly increased cheese yield if many of the milk components normally lost in whey can be incorporated into cheese curd. Microbiological problems are possible because milk is usually concentrated at elevated temperatures to increase the concentration rate. Factors that will affect microbial growth are doubling time of the bacteria, length of time the product is subjected to concentration, growth temperature requirements of the bacteria, and the type of product being concentrated. Evidence indicates that bacterial growth in sweet whey and skim milk can be kept under control using concentration temperatures of at least 48 C if the concentration time does not exceed 6 and 4 h, respectively (20). McDonough and Hargrove (38) reported that ultrafiltration and reverse osmosis equipment can be effectively cleaned and sanitized. They found it essential that equipment be cleaned in place and that the design and operations of the equipment will dictate the cleaning and sanitizing methods used. Abbot et al. (1) concentrated skim milk 2.5-fold by reverse osmosis for 2 h at 30 C. Raw milk increased in bacterial count by a factor of seven due to growth and the concentration effect. However, the same milk treated at 80 C for 20 sec had only a slight increase in bacterial numbers. They concluded that milk could be concentrated without excessive microbiological problems if it were heat-treated, but acknowledged that milk used in their study was of extremely good quality and different results might be obtained with a lower quality milk. Covacevich and Kosikowski (14) examined the growth of bacteria in pasteurized skim milk during concentration by ultrafiltration. They presented data that showed an increase from 42 to 4200 cfu/ml during 4 h of ultrafiltration at 50 C. This was a 16-fold increase in bacterial numbers independent of the concentration factor. However, Mathews et al. (37) concluded that skim milk can be concentrated for cottage cheese manufacture at 4.4 or 49 C without excessive microbial growth. The microbial considerations during ultrafiltration and reverse osmosis have received little attention. If the use of these methods to concentrate or fractionate milk increases, there will be an increase in the microbial problems associated with the technology.

Use of ultra-pasteurization and aseptic packaging of fluid milk products is likely to increase. Many of the cream products are already processed and packaged this way. Heat-stable enzymes produced by bacteria which have been discussed in this paper will continue to be a problem with UHT pasteurized products. It has been demonstrated that these enzymes cause flavor and stability problems in UHT pasteurized fluid products (2, 28,52,67). Mehta (42) has reviewed the literature concerning ultra-pasteurization of milk. Microbiological problems involve the sterilizing effect of the UHT equipment, aseptic condition of packaging, and depending upon the system, the aseptic condition of the environment. Most microbiological problems will be associated with low sterilization temperatures, improper sterilization of the equipment, and contamination during packaging. Research is needed in the packaging area. UHT milk can be packaged aseptically now, but packages with greater consumer acceptability that can be used to package the product without contamination need to be developed.

Potential technologies that will require a microbial evaluation are concentration or heat treatment of milk at the point of production and concentration of milk by freezing or hygroscopic solutions. On-farm concentration might become necessary if energy costs associated with transportation become prohibitive. Possible methods of milk concentration at the farm include reverse osmosis and use of hygroscopic solutions. Before either method can be used, it will be necessary to evaluate cleaning methods (including chemicals used and difficulty), growth of bacteria during the concentration process and process conditions. Such an innovative approach would also require a change in methods used to purchase milk and permit reconstitution of milk at the point of...
processing. Concentration of milk by freezing methods other than freeze-drying will be evaluated. Initial success with this technology would demand an evaluation of the sanitary construction of the equipment used and the potential for microbial growth or microbiologically induced changes in the products.

Excellent progress has been made in the microbiology associated with products made by using cultures. However, problems still exist that are associated with lactic starters. A broad definition of the problem is failure of cultures to produce acid rapidly and uniformly. This can be caused by bacteriophage, agglutination of cultures, antibiotics in the milk supply and loss of protein and lactose metabolizing ability of the cultures. Frozen concentrates of mixed lactic cultures have eliminated the routine transfer and carrying of cultures in the dairy plant and this has reduced the frequency of these problems. Efforts are continually sought to increase the activity of cultures and methods for their production have been reviewed [19]. Frozen concentrates are now available for direct inoculation of vats of milk for cheesemaking. This approach has worked quite well for Cheddar cheese manufacture but problems of uneven acid production and agglutination have been observed in cottage cheese plants. Methods to reduce bacteriophage problems and assure rapid acid production have been directed toward selection of bacteriophage-resistant cultures and development of mixed or multiple-strain cultures [30], selection and development of bacteriophage-resistant starter media [10,23,51] and genetic control of lactic streptococcal protein and lactose metabolizing ability [39,40,41]. Research has been published that shows the rate of bulk starter inoculation does not affect cottage cheese yield [45]. However, cultures that will produce the desired rate of acid development when inoculated at a level of 1% bulk starter need to be developed to manufacture cottage cheese by the short-set method. This will probably result in increased yields for plants using the short-set method and allow plants now using the intermediate- or long-set methods to increase capacity by changing to the short-set method.

**NUTRITION AND HEALTH CONCERNS**

Any evaluation of microbiological problems related to dairy foods must include problems related to the metabolic processes of microorganisms. The typical types of food poisoning and their causes are well understood and can be controlled by proper sanitization, processing and storage techniques. Other problems cannot be controlled as easily.

A problem that is not easily controlled is the production of biologically active amines in dairy foods. These amines are either psychoactive or vasoactive. Psychoactive amines act on neural transmitters while vasoactive amines act on the vascular system [32]. Biologically active amines found in foods have been reviewed by Rice et al. [50]. The amines generally associated with fermented or aged dairy foods are tyramine and histamine. They are formed by enzymatic decarboxylation of the amino acids tyrosine and histidine. The amines are normally oxidized by mono- or diamine oxidases in the body but if they accumulate their biological effects are expressed. A problem arises when the amines are consumed by patients receiving drugs used for treatment of depression. The drugs are often monoamine oxidase inhibitors which block the breakdown of the amines, which then accumulate. Consequently, cheese and many other foods containing biologically active amines have been limited in or removed from the diets of psychiatric patients. Voight and Eitenmiller [63] examined dairy-related bacteria for their tyrosine and histidine decarboxylase activity. They found tyrosine decarboxylase activity in one strain of *Streptococcus lactis,* a *Micrococcus luteus* strain and two strains of *Leuconostoc cremoris*. One strain of each of *Clostridium perfringens* and *Escherichia coli* produced histidine decarboxylase. These same researchers also investigated the relationship between decarboxylase and mono/diamine oxidase activities with the level of amines in cheeses [64]. Dairy-related bacteria generally lacked amine oxidase activities but when it was present, the levels of biologically active amines in cheese were also low. They found decarboxylase activity against tyrosine and histidine in most of the cheeses examined. These authors felt that accumulation of tyramine and histamine was largely controlled by the availability of the free amino acids for decarboxylation. They suggested the development or selection of cultures having oxidase activities for use to control accumulation of biologically active amines in cheeses.

Mycotoxins are toxic metabolites produced by fungi. They can be produced in foods or in the food animals consume. These toxins can cause health problems in humans and animals that are exposed to them. The production, toxicity and occurrence of toxins have been the subject of several reviews [8, 33, 53]. Aflatoxins are of major concern in dairy products because they can be present in milk because of the feed supply. Any product that is made from milk containing aflatoxin will contain the toxin [58,59]. The only method available to control the presence of aflatoxins in dairy products is to control the production of aflatoxins in animal feed or to eliminate the use of contaminated feeds. Mycotoxins can also be a problem for dairy foods due to mold growth during storage. Torrey and Marth [62], Bullerman [7] and Bullerman and Olivigni [9] have detected toxin production by molds that developed on Cheddar and Swiss cheese during refrigerated storage. Several cheese varieties and some other foods are produced with the aid of molds. Increased research efforts to study these molds will undoubtedly result in the identification of more toxins in our foods. Mycotoxins are not a new microbiological problem. They have been the cause of toxicity since the Middle Ages [57]. However, the increased awareness of their potential hazards and current research emphasis on food safety will cause mycotoxin production to be a problem for the dairy industry in the 1980s.

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