Heat Resistant Psychrotrophic Microorganisms

E. B. COLLINS
Department of Food Science and Technology
University of California
Davis 95616

The title contains two relative terms: heat resistant and psychrotrophic. When microorganisms are exposed to a destructive agent such as heat, they die logarithmically. From an understanding of the logarithmic nature of microbial death, any microbial species can survive any given heat treatment if the volume of product is large enough. One species might be so sensitive to a given heat treatment, such as pasteurization, that a large volume of product is required after treatment to contain one living cell—perhaps 4 million liters or more. For another species, survival of the same heat treatment might be 100%. There is a spectrum of differences in heat resistance in between the imaginary species I have used as examples. It is important to keep the numbers of organisms low in a product that is to be heat treated. The smaller the number of organisms in a raw product, the larger will be the total volume required to contain one living organism after the heat treatment is applied. How much spoilage can a milk company tolerate? One liter in 100? One liter in 1000? I understand that in Europe the goal for shelf life of sterilized milk is spoilage of not more than one bottle in one thousand bottles.

The term psychrotroph also is relative. Other terms are used synonymously—terms such as: psychrophile, facultative psychrophile, cold tolerant, or psychrotolerant, depending on the author or speaker. Ingraham and Stokes (7) defined psychophilic bacteria as those that "grow well at 0 C in 2 weeks". Stanier, et al. (16) divide psychrophiles into obligate and facultative psychrophiles and define facultative psychrophiles as "those that can grow at temperatures above 20 C". Witter (19), in a review article published in Journal of Dairy Science, defined psychophilic bacteria as "bacteria that grow at a relatively rapid rate at refrigeration temperatures" and defined refrigeration temperatures as 7.2 C or less. More recently, food microbiologists have started using the term psychrotroph, a name suggested by Eddy (5) for bacteria that grow well at low temperatures. At the October, 1976, meeting of the International Dairy Federation, psychrotrophs were defined as "microorganisms that can grow at 7 C or less, irrespective of optimal temperature".

In this part of the symposium we are concerned with microorganisms that can grow at 7 C or less and that are sufficiently heat resistant to be a problem in pasteurized milk. There are such organisms. Some are members of the genus Clostridium (1). Others are reported to belong to several genera, including Arthrobacter, Microbacterium, Streptococcus, and Corynebacterium (18). Most of them, however, belong to the genus Bacillus. Most of them likely are variants of mesophilic bacilli that have adapted to lower growth temperature ranges. They have been in milk and other dairy products for decades without causing concern. Now they are a problem because money can be saved by extending the shelf life of pasteurized milk as long as possible.

These bacteria cause bitter, fruity, rancid, or yeasty flavors. Some produce sourness. More often they cause sweet curdling. The first manifestation of the defect usually is formation of a sweet curd on the bottom of the milk carton—a defect that might be unnoticed by the consumer. If cartons of homogenized milk are stored 12 days at 7.2 C during early spring and the milk is poured out, about 25% of the cartons will have colonies or buttons about .3 or .6 cm in diameter on the bottom. If cartons of milk are stored 19 or 20 days, about 90% of them will contain buttons.

Mikolajcik and Burgwald (9) in 1953 and Boyd, Smith, and Trout (2) in 1954 observed that thermoduric organisms occasionally grew at temperatures below 10 C, but such organisms generally were uncommon. Psychrotrophic sporeforming bacteria were isolated from soil, mud, and water about 1965 by Larkin and

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Stokes (8) and Sinclair and Stokes (15). That such organisms might be an important problem in milk was emphasized 10 yr ago by a report at the 1969 Annual Meeting of the American Dairy Science Association. Grosskopf and Harper (6) of Ohio State University reported that they had isolated some from milk. Subsequently, studies were done by several investigators.

Heat-resistant, sporeforming bacteria in pasteurized milk are not uncommon. Their presence in milk is not a local problem. It is a potential problem wherever pasteurized milk is stored for more than about 2 wk. In California, Shehata and Collins (13) heated 97 samples of raw milk to 80 °C for 10 min and stored them at 4 to 7 °C for 3 to 4 wk. Species of Bacillus capable of growing at 7 °C or less were in about 30% of the samples—25% of 50 samples collected in the summer; 35% of 47 samples collected in the winter. Chung and Cannon (4) at Auburn University found psychrotrophic sporeforming bacteria in 83% of the raw milk samples they obtained from individual producers. Washam et al. (18) at Oklahoma State University isolated psychrotrophic bacteria from 227 samples of pasteurized milk that had shelf life greater than 20 days at 7.2 °C. Of 700 cultures isolated, 135 were resistant to high-temperature, short-time pasteurization. Most belonged to the genus Bacillus. However, some belonged to other genera: Arthrobacter, Microbacterium, Streptococcus, and Corynebacterium. Overcast and Atmaram (11), at the University of Tennessee, obtained 54 samples of commercially pasteurized milk from dairy plants throughout Tennessee and found 28% developed the sweetcurdling defect in 8 to 10 days at 5 to 7 °C. They isolated and identified some of the causative organisms and found they were Bacillus cereus.

Heat-resistant spore-forming bacteria are in most milk supplies. The numbers likely are small in most cases, but only a few per liter of pasteurized milk can cause spoilage if the milk is stored for 2 to 4 wk under normal refrigeration. Shehata et al. (12) determined the doubling times for several cultures. They varied considerably. Some of those that grew fastest had doubling times of 7.5 to 14 h at 4 °C. Assuming pasteurized milk contains a small number of such bacteria, and assuming it contains one bacterial cell per liter and is stored at 4 °C, how long will be required for spoilage? Calculated from our data, it will require 18 days if spoilage can be detected when there are 50 million cells per milliliter. The time required for spoilage might be shorter or longer than 18 days, depending on initial number, temperature of storage, and growth characteristics of the particular species. Mikolajcik and Simon (10) recently obtained 109 samples of raw milk from producers, heated them 12 min at 80 °C, and stored them at 7 °C. Initially the counts of psychrotrophic sporeformers ranged from less than 1/ml (39% of the samples) up to a maximum of 140/ml. After storage for 14 days, 34% of the samples had counts of more than 1 million/ml. After 28 days, 71% of the samples had counts over 1 million/ml.

It is unlikely that psychrotrophic sporeforming bacteria can be eliminated from the raw milk supply, but there are things that can extend the shelf life of resulting pasteurized milk. What can be done to keep the numbers of psychrotrophic heat-resistant bacteria in milk low? The solution—such as it is—has been with us a long time. Soon after I enrolled in my first course in dairy, I read or was told that the secret to producing good milk is "healthy cows, clean equipment, and good cooling". The last two parts of this solution apply in control of psychrotrophic heat-resistant bacteria. It is important for teats and udders to be clean and dry. Cannon showed that sanitation on the farm was helpful (3). He obtained samples of raw milk from farm tanks, heated them at 80 °C for 10 min, and determined numbers of psychrotrophic sporeforming bacteria with the most probable number method. Numbers in the samples ranged from less than 2 per liter to 240 per liter. Data on line samples indicated that contaminated milking equipment was a problem at some farms. Old gaskets in the pipe lines were a problem at some others. When old gaskets were replaced, numbers of psychrotrophic sporeformers in the milk decreased considerably.

Important also is good refrigeration at the farm, in transit, and at the milk plant. Again, I will use data for the fast-growing strain I mentioned earlier. I will assume that a tank of milk containing bacteria at 10/ml is kept for 48 h. If the storage temperature is 10 °C, the 10 bacteria can become 40,000/ml in 2 days. If the
storage temperature is 2 C, the 10 bacteria will become only 80/ml.

Vegetative cells of psychrotrophic spore-forming bacteria are sensitive to heat or sanitizing agents. Destroying them on equipment at the farm or in the milk plant should be no great problem. But the spores they produce are more resistant (14). Sanitizing with chlorine is probably the best method of destroying spores. Since they are hard to kill, it is important to give due consideration to the various factors that influence resistance of microorganisms to chlorine. It is important for equipment to be clean. Second, it is best to adjust the pH of chlorine solutions to between pH 5 and pH 6. At this pH most of the chlorine will exist as hypochlorous acid. Hypochlorous acid is the molecular species that can enter microorganisms and is responsible for the germicidal action of chlorine. Adjustment to below pH 5 should be avoided; beginning at pH 4, the percentage of free chlorine (Cl2) in solutions increases rapidly as pH is reduced, and this might result in corrosion of equipment. Wang et al. (17) found a sanitizing solution of 50 ppm chlorine adjusted to pH 5 to 6 had about the same germicidal action on spores as a solution of 200 ppm at pH 8. Another factor that will increase the effectiveness of chlorine solution is warmth. Wang et al. found 25 ppm chlorine (pH 5.2) applied at 75 C had about the same germicidal activity on spores as 150 ppm (pH 7) applied at 25 C.

Psychrotrophic bacteria have been a problem in the cold storage of dairy products for a long time. In the past, emphasis has been on heat-sensitive bacteria, including several species, primarily Pseudomonas and Alcaligenes. These Gram negative, nonsporeforming, heat sensitive organisms usually are on equipment and in the milk-plant water supply. Keeping them out of pasteurized milk is not easy. The need for diligence to prevent contaminating milk with them after it is pasteurized has been emphasized often by speakers and in publications. These organisms multiply more rapidly at refrigeration temperatures than do most psychrotrophic sporeformers. I suggest that they still are more important than psychrotrophic sporeformers in limiting the shelf-life of fresh dairy products, including pasteurized milk. Considerable evidence is mounting to indicate that if such bacteria are allowed to reach large populations, the proteolytic and lypolytic enzymes they have produced will survive subsequent heat treatments and spoil products, even though the bacteria that produced the enzymes have been destroyed.

In recent times and as the expected storage life for pasteurized milk has been increased to weeks rather than days, heat resistant psychrotrophic bacteria, especially species of Bacillus, have been added to the list of organisms that milk plants must be concerned about. Control of these sporeformers, similar to the control of nonsporeforming bacteria, requires sanitization procedures at the farm and in the milk plant. It also requires refrigeration at the farm and in the milk plant. But, the sporeformers survive pasteurization, and this is an important difference. Even if contamination after pasteurization were prevented completely, and if all milk were stored at 1 C from cow to consumer, shelf life of milk after it is pasteurized still would be limited to about 3 or 4 wk. This quality is being approached by some milk companies in some areas at the present time.

REFERENCES

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