THE NATURE, SIGNIFICANCE AND CONTROL OF PSYCHROPHILIC BACTERIA IN DAIRY PRODUCTS

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INTRODUCTION

Excellent keeping quality of perishable dairy products has always been the goal of the dairy industry. However, due to certain technological and economic factors, the need for maximum attainment of this property has become of increasing importance in recent years. Storage at low temperatures is a customary by bacterial action; consequently, the existence of a property has become of increasing importance in recent years. The objective of this report is to present a brief discussion of the nature, significance, and control of this group of bacteria. No attempt has been made to cite all literature bearing on this subject. However, pertinent references have been included which will serve as a guide to more detailed information on specific points and to other reports of research.

The term psychrophile\(^1\), as commonly used in the dairy industry, refers to those bacterial species which are capable of relatively rapid growth at low temperatures, generally within the range of 35\(^\circ\)F. (1.7\(^\circ\)C) to 45\(^\circ\)F. (7.2\(^\circ\)C). These are the organisms which are of major importance in affecting the keeping quality of dairy products stored at low temperatures.

TYPES OF ORGANISMS

The organisms belonging to this group are largely Gram-negative non-spore-forming rods. Members of the genera Pseudomonas, Flavobacterium, Proteus, Alkaligenes, Achromobacter, and certain coliforms are most commonly encountered (2) (4) (7) (13) (18).

\(^1\)Presented in part at the Annual Meeting of the International Association of Milk and Food Sanitarians, Inc., at Atlantic City, New Jersey, October 21-23, 1954 as a portion of the report of the Committee on Applied Laboratory Methods.

\(^2\)Psychrophile means cold loving. The term cryophile, now largely discarded, has often been used with identical meaning. However, the prefix "cryo" refers to a more intense cold such as icy or freezing and therefore is a less desirable term.

PSYCHROPHILES IN RAW MILK

Generally, little difficulty is experienced due to the growth of psychrophiles in raw milk supplies for plants located close to their source of supply. Here the milk is processed before appreciable growth takes place.

PSYCHROPHILES IN PASTEURIZED MILK

The importance of psychrophiles in pasteurized fluid milk products is related primarily to keeping quality. A large variety of flavor defects may be attributed to their activity. Some of the more common of these are unclean, putrid, fruity, and an unclean sour odor and taste. Changes in body or appearance often may be observed as a result of their growth, i.e., a thickening often associated with ropy or stringy conditions, or a greenish yellow coloration noticeable usually at the surface. These body and color defects occur almost invariably after flavor defects have become pronounced; they may go unnoticed, however, in products that are frequently agitated as during delivery and transportation.

In recent years increasing amounts of raw milk and cream are being shipped long distances (10). This serves to extend considerably the time that such products are held before processing; thus, sufficient time may be provided during receiving, handling, and transportation of milk to allow very small numbers of psychrophiles initially present to increase until their numbers become objectionable.

Psychrophiles are invariably present to a greater or less extent in all raw milk supplies (3). The extent of their numbers depends upon the sanitary conditions under which milk may be produced and the time which elapses before processing. Since growth of psychrophiles increases as temperature increases, at least to 25\(^\circ\)C. (77\(^\circ\)F.) the temperature of holding will influence their numbers appreciably. Like the coliforms, thermodurics, and thermophiles, they are a part of the normal flora of raw milk, and, like the others, constitute a problem whenever milk is subjected to conditions which favor their eventual appearance in objectionable numbers. Initial contamination may be minimized by good sanitary methods. Where
milk is to be transported long distances, careful attention should be given to the sanitary condition of tank trucks or cans. These must be thoroughly cleaned and sanitized. In this connection it should be emphasized that water supplies, otherwise satisfactory, may be a major source of psychrophiles (7); thus, rinsing of tanks and cans prior to their filling may contribute sufficient of these types so that subsequent growth may become extensive. While the nature of psychrophiles permits their development at low temperatures, these types are retarded markedly (although not as much so as other types) as the temperature approaches the freezing point (5). Consequently, close attention to the maintenance of low temperatures (40° F., preferably lower) during storage and transportation will greatly retard their growth.

Effect of Pasteurization

Most of the available information (1, 12, 16, 20) indicates quite conclusively that proper pasteurization will destroy the psychrophilic bacteria present in raw milk, at least to the extent that the few which may survive would not be a factor in flavor deterioration of properly pasteurized products over an extended storage period.

The influence of psychrophiles on the flavor of milk kept under proper refrigeration does not manifest itself usually until after three or four days of storage and often not until a considerably longer period has elapsed. At a constant temperature of storage, the rapidity with which deterioration takes place will depend largely upon two factors, (a) the initial number of psychrophiles present and (b) the type of organism. The latter factor is of greater importance and accounts for the observation that the total psychrophilic populations found at the time that off flavors of microbic origin occur may be quite variable.

Psychrophiles in Other Pasteurized Products

Several defects of notable economic importance in non-fluid dairy products may be attributed to activities of psychrophilic bacteria. Of primary significance are flavor and physical defects in butter, cottage cheese and solid cheeses. A defect peculiar to butter made from pasteurized cream is commonly identified as "surface taint." A number of different species have been reported to produce the typical condition but it is generally accepted that Pseudomonas putrefaciens is the causative agent (19). Primary stages of "surface taint" are fairly non-specific, varying from loss of typical aroma to an oxidized, "cardboardy" or cooked milk flavor. The more pronounced stages of spoilage will commonly appear within 10 days at storage temperatures of 5° C. (41° F.). As P. putrefaciens is aerobic, spoilage appears initially on surfaces which have free access to air, but the "surface taint" flavor and aroma usually permeate the entire butter mass. Other species of Pseudomonas and Achromobacter as well as members of other psychrophilic genera may be encountered in rancid or cheesey butter (6, 21).

Psychrophilic types also are responsible for widespread losses of cottage cheese. The most common manifestation of psychrophilic activity is a gelatinous, slimy or tapioca curd. Members of several gram-negative genera including Pseudomonas, Proteus, Alcaligenes, Aerobacter and Achromobacter have been associated with the defect (2, 13). Reports have indicated that the predominant organisms may vary with the geographical area involved. Holding temperatures and pH have a decided influence on development of the spoilage. Activities of these species in cottage cheese can be severely limited at pH 4.8 providing the holding temperature is near 5°C. (41°F.). However, at higher temperatures spoilage may take place at pH 4.7 or below. As in the case of butter the loss of typical aroma may precede the more apparent stages of spoilage (14). Bitter, fruity, or unclean flavors also are observed to precede any apparent physical decomposition of the curd particles.

Cheddar and other firm curd cheeses are also affected by psychrophiles. This is especially true in instances where ripening acidities are relatively low. Activity of coliforms in stored cheese frequently results in gas or open curd defects and off flavors. According to at least one report they also act in a synergistic manner, promoting growth of anaerobic butyric acid bacteria in cheese (8). Such stimulation could conceivably result from lowered oxygen tension or increased CO₂ tension as a result of respiration of the aerobic species. Growth of coliforms in cheese usually reaches a peak during the first week of ripening and viable organisms may persist into the second or third month. Open curd defects have also been ascribed to anaerobic sporeformers growing in ripening cheese held at 10° to 12° C. (50° to 53.6° F.) (15). Salt concentrations of 1.7 percent and sufficient acid development may assist in controlling these organisms.

The keeping quality of concentrated milks (3:1 and 4:1) also may be influenced greatly by psychrophiles. Recent studies (11) have shown that psychrophilic growth takes place at an appreciably greater rate in recombined milk than in the concentrate from which it was prepared. Considerable variation in the keeping quality of commercially processed concentrate was observed. This variation was influenced greatly by
the amount and type of post-pasteurization contamination which occurred during manufacture. The psychrophile problem and its control as related to these products is much the same as for fresh milk products.

Methods for Detection

Agar plate method. For selective culture of psychrophiles in freshly pasteurized milk and other products, low temperature incubation is necessary. Recent studies (1, 12) have emphasized this important fact which has been frequently overlooked in the past. Available evidence indicates that organisms which survive pasteurization do not reproduce significantly in milk during storage at 40° to 45°F. (4.4° to 7.2°C.) over a period of one or two weeks; however, growth may be extensive when milk is held at 50°F. (10°C). Likewise, plate counts of fresh pasteurized milk known to be free from post-pasteurization contamination with psychrophiles are almost invariably negative after incubation at 40° or 45°F. for 7 to 10 days. On the other hand, colonies on plates incubated at 50°F. for 7 to 10 days often may be numerous indicating that this temperature allows growth of thermodurics which are not important in the keeping quality of properly refrigerated milk. Consequently, 50°F. is too high an incubation temperature for use in the selective detection of psychrophiles in freshly pasteurized products. In this connection, it has been observed that occasionally no colonies may be found after incubation at 50°F. when milk was examined immediately after pasteurization, but after storage of the milk at 50°F. for three days the counts approached those obtained from plates incubated at 77° and 95°F. (25° and 35°C.) Such observations have been taken to indicate that maximum counts of thermodurics as obtained from plates incubated at 50°F. can only be obtained after the organisms have been allowed a period of adjustment to their environment after being subjected to the heat treatment of pasteurization.

The above shows that the five degrees difference between 45° and 50°F. is critical in the incubation of plates for obtaining psychophile counts, and that counts obtained using an incubation at 50°F. should be interpreted carefully, realizing that such counts may include a significant portion of the thermoduric flora and therefore not give any indication of post-pasteurization contamination with psychrophiles.

Since the presence of psychrophiles is directly related to keeping quality during low temperature storage, the following, based upon available evidence, may be helpful in avoiding misunderstanding and false conclusions:

A. Psychophile counts (incubation of plates at 40° to 45°F. (4.4° to 7.2°C.) for 7 to 10 days) on freshly pasteurized milk usually are very low. Counts obtained at 10°, 25°, 32°, and 35° C. incubation are much higher, as would be expected, due to the growth of non-psychrophilic types.

B. As storage progresses, psychophile, 10° C. and 25° C. counts all increase rapidly, the first gradually approaching the other two, while generally the counts at 32° and especially at 35°C. increase more slowly but do not reach the levels attained at lower incubation temperatures. This is due to the fact that 25° C. is still within the growth temperature range for essentially all psychrophilic types of importance in milk supplies. Some may grow at higher temperatures but a large proportion of them either may not grow or may not form countable colonies; also, 25° C. will allow growth of most non-psychrophilic types; hence, the close relationship between 25°, 32° and 35°C. counts on fresh milk.

C. Bacterial counts of products stored for several days at low temperatures as obtained from plates incubated at 25°C. for 3 days can be expected to include all psychrophiles as well as most other types which may happen to have been present.

D. Mere absence of psychrophiles from one or two milliliters of product (the amount usually examined) is not necessarily a reliable indicator of good keeping quality; however, if detected at all in such amounts of milk, poor keeping quality is almost inevitable (1, 12, 20).

E. When pasteurized milk products are held under refrigerated storage, the longer the storage period the less reliance which can be placed on counts obtained from plates incubated above 25°C.

Other procedures. Recently several other laboratory procedures were studied as possible methods for the detection of psychrophile deterioration of pasteurized milk prior to the development of off flavours (1). The following is a summary of the results of these studies on milk stored at 4.4°C. for a period of 15 days.

A. The resazurin reduction time of pasteurized milk at either 20° or 37°C. was found to be so great that this method was of little help in rapidly obtaining information concerning the activity of psychrophiles.

B. Phosphatase tests were negative throughout the 15 days of storage. In view of this and the diversity of the samples, the possibility of false phosphatase tests occurring as a result of phosphatase production by psychrophiles during storage of properly pasteurized milk was considered extremely remote.

C. The titratable acidity and pH of milk during storage did not change significantly, consequently
these procedures were of no value in following bacterial increases and deterioration of flavor.

D. A marked decrease in protein stability as measured by a protein stability test (17) was closely associated with the development of off-flavour. This test was more valuable in predicting keeping quality than total bacterial counts. This was thought to be due to the fact that population levels associated with flavour changes vary with different organisms.

**CONTROL OF PSYCHROPHILES**

Since proper pasteurization effectively destroys most psychrophiles, their presence in a freshly pasteurized product indicates rather conclusively post-pasteurization contamination of the product. Other faulty practices may be involved but this is the major factor contributing to the presence of psychrophiles in pasteurized products. The effectiveness with which the cleaning and sanitizing procedures are carried out directly influences the number of psychrophiles in finished products. Water supplies used for rinsing purposes may play an important role in such contamination, especially in such products as butter and cottage cheese. However, in the case of pasteurized fluid milk products the available evidence points more directly to the lack of effective cleaning and bactericidal treatment of all equipment surfaces involved beyond the pasteurizer. Since most psychrophiles are markedly sensitive to chlorine, the chlorination of water used in the manufacture of products such as butter and cottage cheese is an effective control measure. In most instances 5-10 ppm available chlorine is considered adequate (9, 13).

The control of the psychrophile problem, therefore involves:

A. Proper pasteurization.

B. Proper cleaning and sanitizing of all equipment, particularly that used following pasteurization.

C. Protection of properly cleaned and sanitized equipment prior to its use.

D. Chlorination, if necessary, of water supplies, especially those used in the manufacture of butter and cottage cheese.

**REFERENCES**


