THE SURVIVAL OF ESCHERICHIA COLI, MICROCOCCUS FLAVUS AND BACILLUS SUBTILIS DURING SPRAY DRYING OF SKIMMILK AND STORAGE OF SKIMMILK POWDER

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ABSTRACT

THE SURVIVAL OF ESCHERICHIA COLI, MICROCOCCUS FLAVUS AND BACILLUS SUBTILIS DURING SPRAY DRYING OF SKIMMILK AND STORAGE OF SKIMMILK POWDER

By

Sterling Samuel Thompson

Investigations were conducted to determine the survival of Escherichia coli, Bacillus subtilis and Micrococcus flavus inoculated into concentrated skimmilk which was spray dried under varying operating conditions. On separate occasions 50 gallons of raw whole milk were separated into skimmilk and cream fractions. The skimmilk fraction was pasteurized at 145 F (62.8 C) for 30 minutes and concentrated to 35-40% total solids in a pilot plant vacuum pan. The approximate ratio of concentration was 4.3:1. A hydrogen peroxide-catalase treatment was used as an adjunct to the pasteurization process. The concentrated skimmilk was heated to 120 F (48.9 C) and sufficient H_2O_2 to give a 0.05% concentration was added to the sample for a contact time of 20-30 minutes. The sample was then cooled to 100 F (37.7 C) and an appropriate amount of sterile catalase was added to decompose the

the H_2O_2 to water and oxygen. Standard plate counts were performed on each sample to determine the bactericidal efficiency of the treatment. The plate counts indicated that H_2O_2 was a beneficial adjunct to pasteurization.

Concentrated milk was inoculated with a pure broth culture concentration of 1×10^6 organisms/ml of Escherichia coli, Bacillus subtilis or Micrococcus flavus and spray dried at three different exit air temperatures, 160, 180 and 200 F (71.1, 82.2, and 93.3 C). While spray drying at various exit air temperatures reduced the numbers of each organism, in no case did it yield bacterial-free powder with the amount of inoculum used. Under all operating conditions B. subtilis was much more resistant to spray drying, followed by M. flavus, and E. coli demonstrated the least resistance.

The skimmilk powders were stored at 25 C (77 F) and evaluated for progressive microbial changes in the product. While substantial reductions were observed with E. coli over 1 to 6 months storage, B. subtilis and M. flavus reductions were less over similar periods of storage.

Spray drying at high temperatures resulted in powders with low moisture contents. This combination of heat and low moisture influenced the survival of E. coli, B. subtilis and M. flavus during drying and storage, however, these factors do not provide absolute microbial control.

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INTRODUCTION

The U.S. Department of Agriculture reported that in 1972, 1,223,456 pounds of nonfat dry milk were manufactured in the United States (55). Nonfat dry milk is used in the preparation of many food products including bread, sausage, ice cream and cottage cheese. Because of its varied usage, it is essential that NFDM be wholesome and free from objectionable bacteria. The bacterial content of NFDM is of concern quantitatively and with respect to the types of microorganisms. Recognition of a relationship between microbial counts on dairy products, such as NFDM, and the care used in production and handling resulted in the establishment of microbiological standards by Federal and state agencies. The microbial content of any food product is usually an index of conditions under which the product was produced and handled, and of the keeping quality.

In general NFDM and other dry milk products are prepared almost exclusively by spray drying. Spray drying at high temperatures reduces the microbial population, but does not render the dried product completely free of microorganisms. Much of the recent research conducted on

microbial populations of spray dried NFDM has been concentrated on the survival of certain pathogenic organisms, Salmonella and Staphylococcus aureus. The literature contails little about the survival of some of the typical contaminants or spoilage type microorganisms.

The objective of this investigation was to determine the effects of various spray drying conditions used in producing milk powder on the viability of Bacillus subtilis, Micrococcus flavus and Escherichia coli.

B. subtilis is a typical aerobic spore forming microorganism which causes spoilage in some dairy products. Hammer and Babel (23) reported that this organism caused coagulation of evaporated milk. Since B. subtilis is a thermoduric microorganism, survival of large numbers of this organism under inadequate heating conditions may cause defects, particularly in reconstituted milks.

Breed et al. (6) indicated that M. flavus is frequently found in milk, other dairy products and on dairy utensils. M. flavus survives conventional pasteurization, and inadequate thermal processing or improper sanitation may cause contamination problems.

Neither B. subtilis nor M. flavus is significant from the standpoint of public health. However, substantial increases in the number of these organisms in NFDM will cause an undesirable increase in total plate count. A higher than acceptable total bacterial count will cause

the failure of NFDM to meet specific grading requirements established by the American Dry Milk Institute.

Traditionally strains of E. coli have been considered indicators of fecal contamination and their presence in dairy products suggests unsanitary conditions or practices during production, processing and/or storage. Hall and Hauser (20) and Insalata (31) suggested that certain serotypes of E. coli may produce food-borne disease. According to Jay (33) enteropathogenic E. coli strains differ from the more normal E. coli strains by being more virulent and by reacting with E. coli OB and O antisera.

Occurrence of outbreaks of gastroenteritis associated with certain E. coli serotypes makes it essential that the food industry become more aware of the potential problems this organism may cause if proper sanitary conditions are not maintained.

Two important factors associated with influencing the microbial content of NFDM are temperatures used prior to drying and method of drying. Frazier (18) indicated that prior to spray drying, milk should be concentrated two or three times and preheated to a temperature ranging from 145 F to 200 F. This preliminary heat treatment would pasteurize the milk, thereby inactivating the less heat stable microorganisms. Other factors which can also influence microbial content are the extent of contamination of the original raw milk, pasteurized milk, processing

equipment, and introduction of contaminants during packaging.

REVIEW OF LITERATURE

In recent years spray dried milk products contaminated with S. aureus enterotoxin have caused several food poisoning outbreaks (2, 4). In addition, contamination of spray dried milk with Salmonella has generated several investigations including those by McDonough and Hargrove (42), Marth (44), LiCari and Potter (38, 39). On the other hand the literature contains little information concerning survival and growth characteristics of contaminants and/or spoilage type microorganisms in spray dried powders during manufacture and storage under either adverse or normal conditions. Therefore, this review will present a general survey of pertinent factors involving the presence of both non-pathogenic and pathogenic microorganisms which have been found in milk powders.

Microorganisms in Milk Powder

According to Jay (33) the microbial content of powdered milk may reach the range of log 6 to 8 per gram. The presence of such high numbers of microorganisms in milk powder would be attributed to the fact that the microorganisms were concentrated on a per gram basis with the milk solids when the water was removed.

Frazier (18) reported that the microbial content of powdered milk depended upon the microbial content of the liquid milk to be dried, the time and temperature of pre-heating, the evaporation process, contamination and growth in storage equipment and the method of drying.

During spray drying a fine mist of concentrated milk under pressure is introduced into a very hot chamber. In general, drying occurs instantaneously at the high drying temperatures. Yet, according to Crossley (10) the fluid milk does not reach sufficient temperatures during drying to insure complete destruction of all non-pathogenic or pathogenic microorganisms.

Some of the initial studies involving the survival of certain non-pathogenic bacteria during drying, post processing contamination and survival of these micro-organisms during storage of dry milk powder were reported by Delepine (1914, cited by Supplee and Ashbaugh, 1922). The investigations indicated that under normal operating conditions the number of microorganisms was 10,000 to 15,000 per gram of powder, as it left the drying chamber. However, recontamination due to subsequent handling caused a sharp increase in the microbial population of the dried powder.

Macy (43) reported that the temperatures employed for commercial spray drying were not sufficient to render a microbial free powder. Macy (43) also reported that a

larger number of microorganisms survived during the spray drying process than during the roller drying process. The higher roller drying temperatures were effective in lowering the bacterial content of the powder. However, these temperatures were less desirable due to the degree of heat damage suffered by the finished product.

Supplee and Ashbaugh (51) observed that regardless of the number of bacteria present in the liquid milk, the number which survived drying was low and did not reflect a direct relation to the number of bacteria in the liquid milk prior to drying, if the milk contained the normal flora. Their investigation demonstrated that any increase in microbial content was largely due to recontamination subsequent to drying. Crossley and Johnson (12), during their investigations of the microbial content of milk powder from two separate processing plants, also observed a lack of relationship between total counts on raw milk and powdered milk. However, they concluded that the microbiological quality of the powdered milk depended ultimately upon the numbers and species of organisms which survived pasteurization. The drying temperatures employed caused some bacterial destruction.

These early investigations and later investigations by Higginbottom (24, 27, 28), Crossley (9), Findlay et al. (16) and Olson and Nielson (47) established precedence for some of the more recent research regarding the survival of

non-pathogenic as well as pathogenic microorganisms during the manufacture and storage of spray dried milks.

Microbiological Standards for Dried Milk Powder

Microbial standards for grades of nonfat dry milk and powdered whole milk were established by the American Dry Milk Institute and published in Standards for Grades of Dry Milks (1). According to Ingram (30) bacterial standards serve three functions: (a) minimize the risk from pathogenic organisms, (b) insure that the product was not grossly contaminated, and (c) give an estimate of product shelf-life during storage. Davis (14) reiterated the desirability of microbial standards by enumerating the following advantages:

a. insure a wholesome safe product for human consumption.
b. insure adequate keeping quality.
c. indicate points of faulty processing during operation.
d. improve the quality of the product.
e. educate workers in hygiene and other aspects of their work.

Data in Table 1 lists the bacterial standards for some dried milks.

Table 1.--Specific bacterial grading requirements for dried milk (1).

Type and grade of powder	Bacterial estimate per gram	
	Spray Not Greater Than	Atmospheric Roller Not Greater Than
Nonfat Dry Milk		
Extra	50,000/g	50,000/g
Standard	100,000/g	100,000/g
Dry Whole Milk	Gas Packed	
Premium	30,000/g	
Extra	50,000/g	
	Bulk	
Extra	50,000/g	
Standard	100,000/g	
		Bulk
Extra		50,000/g
Standard		100,000/g
Instant NFDM	30,000/g	

Note: The presumptive Coliform count on dry milk products should not exceed 90 per gram and except for instant nonfat dry milk, it should not exceed 10 per gram. No pathogens or their toxin should be present.

Types of Microorganisms in Milk Powder

Higginbottom (27) suggested that due to the increased production and use of dried milk, researchers should not only be concerned with the number of bacteria but also with the type of bacteria in milk powder, especially those which grow readily after reconstitution. There existed the possibility that the reconstituted milk might be held for some extended time prior to use and the inference that certain milk-containing foods were responsible for food poisoning made it essential that the types of organisms which survived the drying process be known.

In a review of literature prior to 1949 dealing specifically with the relationship between production procedures and microbial population of milk powder, Crossley and Mattick (13) concluded that the microflora of spray powder was directly related to the preheating temperature of the initial liquid milk, the concentrated milk, equipment and plant cleanliness.

Foster et al. (17) reported that the use of high preheating temperatures was instrumental in reducing the number of thermoduric microorganisms in the final powder. Streptococci and aerobic spore formers predominated. Preheating at lower temperatures resulted in the survival of large numbers of micrococci and microbacteria. The data indicated that no pathogenic organisms survived processing. Later this evidence was proven to be incorrect by the

research of McDonough and Hargrove (42), LiCari and Potter (38), Anderson and Stone (2) and Armijo et al. (4) in relation to the presence of Salmonellae and S. aureus in nonfat dry milk.

Observations by Cihova and Saxl (8) demonstrated that although the number of organisms isolated from dried powder processed at three separate plants differed, the types of organisms were in fact similar. B. subtilis, B. licheniformis, B. pumilus, B. cereus, B. megatherium and B. alvei occurred more frequently among the sporulating flora isolated, whereas gram positive cocci, particularly S. faecium were the predominating organisms of the total powder flora.

Keogh (37) theorized that the degree or level of lactic acid or lactate in milk powder was a rough indication of the number of lactic acid producing organisms in milk. (Lactic acid is thermally stable at the various heat treatments used.) In previous studies it was observed that the heat treatment the milk received during the processing of NFDM was not sufficient to eliminate all bacteria. However, unlike past assumptions that the presence of the less heat resistant bacteria in milk powder was due to post processing contamination, it was suggested that the heat sensitive organisms were in the powder because the protein in the milk protected these organisms from the heat and that actual temperature of the particles

was less than indicated in the drying chamber. Bacteriological analysis of the powder demonstrated that the flora consisted mainly of spore formers and other thermoduric types such as micrococci and microbacteria. Some pathogenic organisms were also isolated including S. aureus, S. pyogenes and C. perfringens and their presence is especially undesirable if the powder is incorporated as an additive to other foods.

At the Sixth International Symposium of Food Microbiology, Planine and Milohnoja (48) presented data on one of the more recent investigations concerning the microbial content of milk powder. By analysis of variance they illustrated that daily powder samples differed in bacteriological contamination, which was attributed to the varied degrees of microbial contamination of the raw milk. The organisms which were isolated included fecal streptococci, coliforms, sulphite producing clostridia, S. aureus, thermophilic and other thermoduric organisms, psychrophilic, acidophilic, caseolytic and lipolytic bacteria. A few yeasts and molds were isolated, however, no Salmonellae were detected.

Galesloot and Stadhouders (19), at the same symposium, presented a related paper in which it was theorized that the presence of certain types of bacteria in dried milk products originated from three primary sources.

a. Raw milk. The heat treatment that the raw milk was subjected to during processing was not sufficient to kill all of the bacteria present. Only the thermoduric organisms survived pasteurization; Microbacterium lacticum was the most heat stable.

b. Growth of organisms during the process. The entire process was conducive to bacterial growth. Growth during the process was observed with mesophilic and thermophilic microorganisms. Group D streptococci made up the major portion of the mesophilic bacteria with S. durans the dominant species. B. stearothermophilus var. calidolactis was the dominant thermophilic organism. On a few occasions S. aureus was observed.

c. Incidental contamination. Contamination with microorganisms which did not grow during the process and caused low level contamination. The major causes of incidental contamination were related to direct human contact, air borne contamination during drying, cooling, transporting, instantizing and packaging. The Enterobacteriaceae (Coliform bacteria, Salmonellae) and S. aureus comprised the organisms responsible for that type of contamination. These organisms which originated from incidental contamination were not related to plant conditions at the time of

processing but rather to the types in the processing plant.

Taha et al. (52) in Part I of a dual investigation studied the microbiological quality of spray dried milk obtained from a specific processing plant. A total bacterial count using two sets of plates with one set incubated at 30 C (86 F) and the second set at 37 C (98.6 F) showed average counts of 13 x 10^6/gram and 6.8 x 10^6/gram, respectively. Thermoduric plates yielded an average count of 1.6 x 10^6/gram. This high count was attributed to the presence of large numbers of heat resistant organisms in the raw milk and to contamination during processing. Psychrophilic counts averaged 4.5 x 10^5/gram. Heat treatment destroyed these organisms during the process, thus, their presence in the powder was due to post processing contamination. Non-pathogenic staphylococcal counts averaged 4 x 10^5/gram. Since these organisms were quite heat labile their presence was attributed to post processing contamination. Coliforms showed an average count of 6.4 x 10^2/grams and were isolated from 20% of the samples. Since they too were found to exist exclusively as non-heat resistant strains, their presence was construed to be due to post processing contamination also. Group D streptococci were divided into two groups. Group I included S. faecium, S. durans and S. bovis, and showed an average count of 5.3 x 10^5/gram. Group II consisted of S.

faecalis, S. zymogenes and S. liquefaciens, with an average count of 9.3×10^4/gram. The presence of both groups of streptococci was attributed to their relatively high resistance to heating and drying and to recontamination following pasteurization and drying. Saccharolytic anaerobes were found in 40% of the samples, no exact number was specified.

In the second half of the investigation Naguib et al. (46) identified more of the predominating microorganisms in the spray dried milk samples. The organisms isolated, in decreasing order, were streptococci, micrococci, microbacteria and sporeformers. Five hundred ninety-eight cultures were isolated from plates which were incubated at 30 C (86 F) and 37 C (98.6 F). Organisms isolated from plates incubated at 30 C were 67.2% streptococci, 19.6% micrococci, 7.4% microbacteria, 3.4% spore forming bacilli and 2.4% were Sarcina. Organisms isolated from plates incubated at 37 C were 72.9% streptococci, 12.9% micrococci, 8.9% microbacteria and 5.3% spore forming bacilli.

Although several of the previously mentioned investigations indicated that adequate preheating of liquid milk and efficient drying temperatures eliminated the possibility of any pathogenic organisms surviving, there have been several outbreaks associated with staphylococcal food poisoning (2, 4, 11, 29, 37). A few food

poisoning outbreaks have also been associated with Salmonellae and as a result several investigations were conducted with reference to the heat resistance of these organisms to spray drying and the effects of storage on their survival in nonfat dry milk. McDonough and Hargrove (42) concluded that although certain combinations of heat and moisture were sufficient in reducing the probability of Salmonellae survival during spray drying, these factors cannot be relied on for complete control. Adequate pasteurization destroyed Salmonellae in liquid milk, however, much higher temperatures were required to completely destroy these organisms in concentrates. According to Julseth and Diebel (35) 15,000 to 30,000 Salmonellae per gram were sufficient to evoke a reaction in infants and adults, respectively. LiCari and Potter (38) reported that spray drying at commercial temperatures killed substantial numbers of Salmonellae in skimmilk, but under no conditions did the treatment render the powder completely free of Salmonellae.

These investigations clearly established the fact that pathogens and their toxins survived the spray drying operation, as evidenced by the number of outbreaks associated with them. Therefore, low numbers of these organisms should be significant since these numbers will increase rapidly with hydration and incubation.

Changes in Microbial Content During Storage

According to Haines and Elliot (22) the rate of microbial die off in milk powder was influenced by moisture content, temperature and nature of the micro-organisms present.

The amount of moisture in milk powder is directly related to its keeping quality because many microorganisms are incapable of product spoilage at low water content. As early as 1922 Supplee and Ashbaugh (51) observed that even if the microbial population of the powdered milk was significantly high, any relationship between bacterial numbers and keeping quality was eliminated by the lack of sufficient moisture to allow propagation. Later studies confirmed their observations. Foster et al. (17) reported that low moisture levels prevented microbial metabolism, thereby eliminating microbial spoilage of dry milk. During the storage of roller dried milk the total microbial population decreased rapidly during the first month but became relatively constant after two to four months. A similar decrease was observed with spray dried milk during storage, however, the die-off was less marked. Spore formers and micrococci tended to survive longer than most other micro-organisms.

Keogh (36) and Brockman (7) reported that micro-organisms which survived spray drying did not grow in the powder if the initial moisture level was low and if the

powder was protected from absorbing more moisture. Keogh suggested that microbial die-off during storage was attributed to the oxidation of enzymes. However, Brockman (7) suggested that the bacteriological quality or stability of the product which was stabilized by reduction in moisture content resulted from an interruption of processes necessary for microbial growth.

Higginbottom (28) discussed the effect of relative humidity on bacterial numbers during storage and concluded that at relative humidities of 80-100% there was a rapid decrease in bacterial numbers followed by rapid growth of bacteria and molds. Relative humidities below 80% caused an increase in microbial population with maximum survival at approximately 10%.

Data in Table 2 lists the moisture standards for milk powders.

Decreases in microbial populations in milk powder stored at room temperature over prolonged storage were observed by Crossley and Johnson (12). In addition they observed that the rate of microbial reduction was accelerated by higher storage temperatures.

McDonough and Hargrove (42) investigated the effect of moisture, temperature and length of storage on the survival of Salmonellae. The survival of these organisms was directly influenced by temperature. No significant decrease in population was observed in powders stored

Table 2.--Specific moisture standards for milk powders (1).

Powder and grade	Moisture content	
	Spray Process Not Greater Than	Atmospheric Roller Not Greater Than
NFDM		
Extra	4.00%	4.00%
Standard	5.00%	5.00%
Dry Whole Milk	Gas Packed	
Premium	2.25%	
Extra	2.50%	
	Bulk	
Extra	2.50%	
Standard	3.00%	
		Bulk
Extra		3.00%
Standard		4.00%
Instant NFDM	4.50%	

at 26.6 C (79.8 F), however, as the temperature increased to 37.7 C (99.8 F), 43.3 C (109.9 F) and 50 C (122 F) the rate of destruction likewise increased. Although the higher storage temperature caused a decrease in microbial population, it proved to be undesirable due to the development of objectionable flavors. Survival of these organisms was also related to moisture content. A decrease in viable organisms occurred up to 15 to 20% moisture, above 20% destruction leveled off, and at 40% moisture, microbial growth was observed.

LiCari and Potter (39) in a more recent study of microbial survival during drying and storage of nonfat dry

milk considered Salmonellae survival in powders incubated at 25 C (77 F) to 55 C (131 F). In four to eight weeks at 45 C (113 F) and 55 C there was a three or more log cycle reduction observed. However, at 55 C adverse flavors in addition to other physical defects were observed after one week storage. Storage at 25 C and 35 C (95 F) caused a much slower rate of destruction. In general, microbial die-off occurred at a dual rate with rapid destruction during the first two weeks of storage followed by relatively lower destruction rates.

Bibek et al. (5) confirmed the work of LiCari and Potter. Their study showed that death rates, measured as total numbers, were quite high during the first two months of storage. Survival of these organisms in nonfat dry milk was considered to depend on five factors:

a. Initial number of organisms present
b. Strain of organism
c. Temperature used during process
d. Kind of product manufactured
e. Conditions and duration of storage.

During storage of the contaminated powders, different organisms exhibited different survival rates with some much more resistant to storage conditions than others.

According to Crossley (10) decreases in the number of organisms in several powdered milk samples varied considerably between the powders. Some decreased 50% after

one month, in others reduction was relatively small even after six months. The author concluded that bacterial reduction depended primarily upon the nature of the flora. Spores survived for long periods, but streptococci died quite rapidly.

By analysis of variance, Planine and Milohnoja (48) reported that the number of aerobic and facultative organisms per gram of powder decreased parabolically during 12 weeks of storage. Fecal streptococci declined from 300 to 170 organisms/g, thermophiles declined from 92,000 to 31,000/g, thermodurics declined from 103,000 to 40,000/g and psychrophilic bacteria declined from 44,000 to 17,000/g. After four weeks of storage 34% of the samples met the desired microbial standards, after eight weeks 59.6%, and finally after twelve weeks 68% met the standards.

Significance of Microbial Counts on Milk Powder

Since utilization of nonfat dry milk as a food additive has increased over recent years, it is essential that it be wholesome and free from any objectionable bacteria. Even though it has been demonstrated that the number of organisms which survived spray drying decreased during subsequent storage, small numbers in milk powder proliferate rapidly upon hydration and incubation. Relatively low numbers of non-pathogenic or pathogenic

organisms introduced into a food product which was not heat treated or received an insufficient heat treatment prior to consumption can be significant, as evidenced by physical defects in the product or association with food poisoning outbreaks.

Mattick et al. (45) suggested that plate counts on milk powder were related to plant cleanliness and sterility, preheating temperatures and bacteriological quality of the raw milk. Following this investigation Findlay et al. (16) also observed that bacterial counts on spray dried powder were directly related to the preheating temperatures applied to raw milk and condensed milk. Counts were low when preheating temperatures of 190 F (87.7 C) and 200 F (93.3 C) were used. Counts were only slightly higher when 180 F (82.2 C) was used, but at 160 F (71.1 C) and 170 F (76.6 C) the counts were relatively higher.

Von Loesecke (57) and Davis (14) both concluded that spray powders with low microbial populations were an indication of proper manufacturing practices, utilization of clean raw milk and proper storage following drying. Organisms which were present in the dry powder were those which survived forewarming and drying, or those introduced while packaging. Only the more resistant organisms were able to survive high temperatures during drum drying, whereas with spray drying, the less resistant forms survived the process. Emphasis was placed on the theory that

the microbial content of dry milk furnished an index of product purity. Although Hammer and Babel (23) agreed that low bacterial counts indicated thorough heating during processing and adequate protective measures against contamination, they disagreed with the finding that the bacteriological quality of the raw milk influenced the bacteriological quality of the final powder. They deduced that plate counts on dry milk, whether high or low, could not be influenced by the microbiological quality of the original raw milk, since there was substantial microbial destruction during normal processing.

Foster et al. (17) and later Crossley (10) reported that the presence of coliforms in milk powder as in other pasteurized dairy products suggested unsanitary conditions and practices during production and storage. Isolation of molds from dry milk indicated excessive air contamination or poor handling during the process.

McDivitt et al. (41) and Hall and Hedrick (21) suggested that although the total bacterial count on dry milk may be low or the number of pathogens present is low or undetectable, this does not insure the safe quality of the product. Pathogenic staphylococci isolated from dry milks, even in low numbers, constitute a health hazard, especially if the product is held improperly after reconstitution. The enterotoxin produced by these organisms

is not destroyed by the temperatures commonly used in processing spray dried milk.

Control of the Microbial Content of Dried Milk

According to Keogh (36) the microbial content of milk powder was influenced more by the number of thermoduric organisms in the raw milk than by the total bacterial content of the initial raw milk since those organisms were capable of surviving the temperatures used during processing.

A review of the literature indicates that using high preheating temperatures has the most dramatic effect on controlling microbial populations of milk powder. However, there also must be good combinations of adequate plant and equipment sanitation, avoidance of air borne contamination, proper post processing and handling procedures, selection of adequate storage containers and proper storage conditions.

Galesloot and Stadhouders (19) suggested a few specific measures which could be taken in order to control the microbial content of dry milk. They suggested better control of conditions during milk production at the dairy farm in order to control the number of thermoduric organisms which originated in the raw milk. Raising the pasteurization temperature was useful in controlling those thermoduric bacteria obtained from the dairy plant.

Bacterial counts may increase in the milk as it is moved from the pasteurizer to the drier. Consequently, adequate precautions should be taken to prevent the equipment involved during this process from becoming a continuous culture apparatus. Large balance tanks should not be used and capacities of the different sections of the installation should be in appropriate relationship to each other. The length of storage time should be short and the use of film evaporators in preference to circulation evaporators was suggested. Direct human contact with the milk during any part of the processing should be avoided. Concentrated milk should be pasteurized before it is pumped to the drier. This should control the thermoduric population in the powder. The final drying temperature was less effective for bacterial destruction than minimum pasteurization, therefore it had a limited effect on controlling the microbial content of the powder. Organisms which developed in the process before drying were mostly thermoduric organisms with a few non-thermoduric organisms such as S. aureus and Enterobacteriaceae. Normally these non-thermoduric organisms are destroyed during the drying operation. Finally, air-borne contamination during drying should be prevented. A drier which operates under negative pressure utilizes outside air. Any bacteria which may be present in the outside air will not be heated to the same degree as the organisms which were present in

the concentrated milk, thus they have an increased chance of surviving. In order to prevent air borne contamination the authors suggested that air within the plant should not be used.

Effect of Spray Drying on Various Microorganisms

Crossley and Johnson (12) observed that high drying temperatures, 165 C (329 F) and above caused considerable bacterial destruction. The investigation stressed the significance of employing the highest possible temperature without causing severe physical damage to the product in order to obtain relatively high thermal efficiency. Spray drying proved to be quite destructive to many bacteria. However, on the basis that some non-thermoduric organisms were able to survive drying, it was concluded that spray drying could not be relied on to eliminate pathogens. Crossley (10) observed that different organisms varied in their susceptibility to various spray drying conditions. He observed an increase in microbial survival when the inlet air temperature fell below 311 F (155 C) and a decrease in survival when the inlet air temperature was above 330 F (165.5 C). However, even when the inlet air temperature was raised to 410 F (210 C) some non-spore forming organisms survived spray drying.

According to Jay (33) most microorganisms were destroyed during drying, however bacterial endospores

survived spray drying as did some yeasts, molds and some gram positive and negative bacteria.

According to Hammer and Babel (23) spray drying caused a rapid loss of moisture during dehydration which kept the temperatures so low that it permitted survival of some of the more heat labile organisms. Generally the number of organisms which survived spray drying was relatively low. Those organisms were either believed to be protected in some manner that was not applicable to those organisms which were destroyed, or they were much more heat resistant. Foster et al. (17) concluded that this protective effect was caused by a layer of dried milk solids which remained around the bacterial cell, thus preventing complete dessication. Keogh (36) concluded that the protein protected these heat sensitive organisms from the temperatures used during spray drying.

LiCari and Potter (38) while working with Salmonellae in nonfat dry milk suggested that the inlet air temperatures from 176 C (348.8 F) to 232 C (449.6 F) be used to spray dry nonfat dry milk. Exit air temperature dropped, in some instances, below 93 C (199.4 F). Product temperatures, however, were not maintained at this exit air temperature throughout drying due to cooling of the evaporated water. Consequently, during drying most organisms were dehydrated below lethal temperatures and as soon as they were dried they became more resistant to the

temperatures that were used. In addition to being relatively resistant to those temperatures the organisms were assumed to be protected from heat by the milk solids. The microorganisms which survived spray drying were organisms which entered the product via the evaporator, contaminated air or a dirty drier.

MATERIALS AND METHODS

Selection and Propagation of Microorganisms

Stock cultures of E. coli, B. subtilis, and M. flavus were obtained from the Department of Food Science and Human Nutrition, Michigan State University. E. coli was maintained in Nutrient Broth (Difco Laboratories, Detroit, MI), B. subtilis and M. flavus were maintained in Trypticase Soy Broth (BBL, Division of Becton, Dickinson and Co., Cockeysville, MD). The stock cultures were stored at 4 C (39.2 F). Three days prior to the inoculation of E. coli, B. subtilis or M. flavus into the concentrated milk, one milliliter of the pure culture was transferred to 99 milliliters of Nutrient Broth or Trypticase Soy Broth. The inoculated broth was then incubated at controlled temperatures in a NBS Gyrotory Incubator Shaker (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey). Broth cultures of M. flavus were held at 25 C and broth cultures of E. coli and B. subtilis were held at 35 C. (Agitation of these broth cultures caused a marked increase in microbial population over non-agitation.)

The active broth culture of the appropriate organism was transferred every 24 hours for three days prior to inoculation of the milk. After the first 24 hour incubation period, the culture was diluted in phosphate buffer dilution blanks. The 24 hour cultures were plated according to the recommended Standard Plate count method in Standard Methods for the Examination of Dairy Products (3) with E. coli on Violet Red Bile (VRB) Agar and M. flavus and B. subtilis on Plate Count Agar (PCA) (Difco) in order to determine the approximate population of each culture. Using these plating data as a basis, a sufficient amount of culture was inoculated into the milk to obtain the desired concentration of 1×10^6 organisms/milliliter.

Preparation of Concentrated Skimmilk

Prior to each spray drying run, 50 gallons of raw whole milk were obtained from the Michigan State University Dairy Plant. The milk was separated, concentrated, and the skimmilk fraction was eventually subjected to a hydrogen peroxide (H_2O_2)-catalase treatment (58) as an adjunct to pasteurization in order to substantially reduce the number of surviving bacteria in the pasteurized skimmilk.

According to Hall and Hedrick (21) separation of cream can be accomplished with or without preheating the milk. Preheating milk to 68 to 86 F (20-30 C) enhances

efficiency of separation, but since there was a short holding period during processing, cold milk separation was more practical. The raw milk was separated into skimmilk and cream fractions using a DeLaval Air Tight Cream Separator (The DeLaval Separator Co., Poughkeepsie, NY). The skimmilk fraction was then pasteurized at 145 F (62.8 C) for 30 minutes.

By utilizing a direct pipeline hook-up system, the pasteurized skimmilk was pumped from the vat and condensed in a vacuum evaporator (C. E. Rogers, Detroit, MI) to 35-40% total solids. During evaporation the percent total solids was determined by using a Baumé hydrometer (Fisher Scientific Company, Detroit, MI). Once the desired total solids was obtained the condensed skimmilk was drained from the vacuum pan into a sterilized ten gallon milk container. A chart converting Baumé readings to total solids was used to calculate the final percent total solids (21).

Immediately following evaporation, the skimmilk fraction was cooled to 45-42 F (7.2-5.5 C) and placed in a walk-in cooler at 3.3 C (38 F) overnight.

Hydrogen Peroxide-Catalase Treatment

The germicidal characteristics of H_2O_2 have been known since its discovery by Thenard in 1818. In 1883, Schrodt established the use of H_2O_2 for preserving milk.

Since that time, numerous publications have been submitted relating to this subject.

Luck (40) suggested that using H_2O_2 in treating milk served two purposes:

a. Substitute short time treatment in place of pasteurization by heat, thus reducing the total bacterial count, and
b. preservative to maintain the keeping quality for a longer period.

He also suggested that treatment of milk with H_2O_2 caused a higher bacterial reduction than pasteurization by heat. According to Roundy (50) treating milk with H_2O_2 caused a selective destruction of many of the undesirable bacteria without adversely affecting the milk itself. The effectiveness of the H_2O_2 process depends on the temperature of the milk, the bacteriological quality of the milk at the time of sterilization, the concentration of H_2O_2 used, and the duration of the treatment. Luck (40), Roundy (50), and Walker and Harmon (58) found that adding 0.05% H_2O_2 to milk and heating to 120 F (48.9 C) established effective bactericidal conditions.

In this investigation an 8.5-10 gallon sample of pasteurized, condensed skimmilk was heated in a steam-water jacketed kettle to 120 F (48.9 C) and treated with a 0.05% concentration of H_2O_2 (Mallinckrodt Chemical Works, St. Louis, MO) for a period of 20 to 30 minutes.

At the end of the 20 to 30 minute contact time, the milk was cooled to 100 F (37.7 C). It was essential that all of the H_2O_2 added to the milk be decomposed before adding the appropriate pure culture and this was accomplished by adding sterile catalase (Nutritional Biochemicals Corporation, Cleveland, Ohio). Studies by Luck (40), Underkofler (54), Walker and Harmon (58) show that cooling milk after the H_2O_2-heat treatment enables the catalase to function more efficiently in breaking down H_2O_2 to water and oxygen. According to Roundy (50) adding excess catalase was not harmful. Four to five times the conceptual amount of catalase needed to destroy the H_2O_2, diluted with at least five times its volume of sterile water, was used. The milk was well agitated during the addition of H_2O_2 and catalase. In order to ensure that all of the residual H_2O_2 was decomposed by the catalase, a few drops of freshly prepared 25% solution of potassium iodide and 2% soluble starch solutions were added to two 5 ml samples of milk, one treated the other untreated. The resulting colors were compared; identical colors in the treated and untreated samples indicated complete destruction of H_2O_2 (50). In most instances the H_2O_2-catalase treated sample showed a yellow discoloration, indicating that the residual H_2O_2 had not been decomposed. Based on this, the test was repeated at five minute intervals until no color change was noted in the treated sample. In the presence of

H_2O_2, a solution containing potassium iodide turns yellow due to the liberation of free iodine.

At the end of the H_2O_2-catalase sterilization treatment, a sample of the treated milk was plated on PCA and the plates incubated in order to evaluate the effectiveness of the treatment in reducing the number of bacteria which survived pasteurization. An investigation by Roundy (50) indicated that aerobic spore forming organisms are more resistant to destruction by H_2O_2, the coliform organisms are the least resistant and the susceptibility of certain lactic acid organisms lies somewhere in between. According to Ito et al. (32), aerobic spore formers, e.g., Bacillus subtilis globigii and Bacillus polymyxa were more resistant to H_2O_2 than anaerobic spore formers Clostridium sporogenes or Clostridium botulinum, with the exception of C. botulinum type B.

Spray Drying

A vertical down-draft, direct gas fired, stainless steel spray dryer, manufactured by the Marriott Walker Corporation of Birmingham, Michigan was used. Three different exit air temperatures, 200 F, 180 F and 160 F (93.3, 82.2 and 71.1 C) were used to study the effects of drying temperatures on microbial destruction. On separate occasions a 1×10^6 concentration of the appropriate culture was inoculated into the concentrated milk a few minutes before drying. The concentrated skimmilk had an

approximate temperature of 100 F (37.7 C). One third of the mixture was added and dried at each exit air temperature, beginning with the higher temperature. The liquid sample was not added until equilibrium drying conditions were achieved at the desired outlet temperature. Samples were pumped by a high pressure pump to the top of the drier and atomized through a high pressure spraying nozzle into the chamber of the drier.

A representative portion of each powder which had been dried at the specified temperature was collected asceptically in a large sterile glass jar. Since storage stability is an important factor in processing practice, the glass jars served as unique storage containers. The jars are impermeable to moisture vapor, consequently, they prevent absorption of moisture during storage. Absorption of moisture could very easily influence product deterioration due to chemical and bacterial changes (21).

The following table gives the drying conditions used for each trial.

Moisture Determinations

The percent moisture in each dry milk sample was determined by the Karl Fischer titration, using a Beckman Model KF-2 Aquameter equipped with a duo-platinum electrode (Beckman Instruments, Inc., Scientific and Process Instruments Div., Fullerton, Cal.). The Karl Fischer reagent (Fisher Scientific Company, Detroit, MI) reacts

Table 3.--Drying conditions used to prepare skimmilk powder.

A. Organism: *M. flavus* % Total solids - 35%

Nozzle:

Type	SX Insert	65 Core	17A
Homo pressure (psi)	1200	2300	2600
Pump speed	1.8	3.2	3.3
Air damper setting	12.7	12.7	12.7
Gas pressure	5.0	3.0	3.9
ASME nozzle	1.3	1.3	1.3
Inlet air, F	275	262	240
Exit air, F	200	180	160
Ambient air, F	78	78	78

B. Organism: *B. subtilis* % Total solids - 37%

Nozzle:

Type	SX Insert	65 Core	17A
Homo pressure (psi)	1000	2100	2550
Pump speed	0.90	3.2	3.5
Air damper setting	13.4	13.4	13.4
Gas pressure	4.4	4.6	4.4
ASME nozzle	1.3	1.3	1.3
Inlet air, F	325	310	295
Exit air, F	200	180	160
Ambient air, F	75	75	75

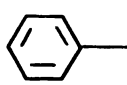
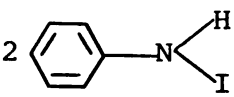
C. Organism: *E. coli* % Total solids - 40%

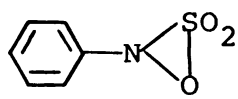
Nozzle:

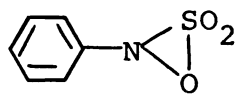
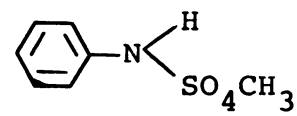
Type	SX Insert	65 Core	17A
Homo pressure (psi)	1150	2300	3000
Pump speed	1.0	3.0	3.6
Air damper setting	13	13	13
Gas pressure	4.0	4.0	3.5
ASME nozzle	1.5	1.5	1.5
Inlet air, F	288	285	265
Exit air, F	200	180	160
Ambient air, F	75	75	75

quantitatively with the water in the sample to give a sharp chemical change (titration endpoint) that is detected electrochemically by the aquameter. Anhydrous methanol (Mallinckrodt) (active hydrogen compound) used to extract the moisture makes the entire reaction water specific, and in addition it acts as a stabilizer for the reaction.

According to Joslyn (34) the following oxidation-reduction occurs in two steps:

(1) $I_2 + SO_2 + 3$  $+ H_2O \longrightarrow 2$  +



(2)  $+ CH_3OH \longrightarrow$ 

Sodium tartrate dihydrate ($Na_2C_4H_4O_6 \cdot 2H_2O$) which contains 15.66% water under normal laboratory conditions was used as the primary standard for determining the titer of the Karl Fischer reagent (53).

The following formula demonstrates the method of obtaining percent moisture:

Standardization:

(3) mg H_2O/ml Karl Fischer reagent =

$$\frac{\text{weight of } Na_2C_4H_4O_6 \cdot 2H_2O \times 0.1566}{\text{ml of K. F. reagent}}$$

Moisture content:

(4) % Moisture =

$$\frac{\text{ml of K. F. reagent} \times \text{mg } H_2O\text{/ml K. F.}}{\text{weight of dry milk sample}} \times 100$$

The Karl Fischer titration is applicable to all organic compounds and reproducibility is ± 0.05 milliliter of reagent (53).

Microbiological Analyses of Skimmilk Powder

All of the dry milk samples were microbiologically analyzed according to the methods described in Standards for Grades of Dry Milk, American Dry Milk Institute (1), and Standard Methods for the Examination of Dairy Products (3). Since no difficulty occurred with undissolved particles, phosphate buffered distilled water was used rather than 1.25% sodium citrate dilution blanks. The dilution blanks contained several small glass beads which facilitated particle break up and solubility.

Within two to four hours following spray drying, a representative sample of each powder was plated on the designated media to determine the estimated number of the initial population which survived the three different spray drying temperatures. Samples initially inoculated with E. coli were plated on VRB agar and incubated at 35 C (95 F) for 24 ± 3 hours. Samples initially inoculated with M. flavus or B. subtilis were plated on PCA and incubated at 25 C (77 F) and 35 C (95 F) for 48 hours, ± 3 hours.

All samples were stored at 25 C (77 F) in large glass jars, in order to determine what effect length of

storage and storage temperature would have on the microbial population. Each sample was microbiologically analyzed every week for the first six weeks and biweekly, thereafter.

RESULTS

Microbiological Analyses of Concentrated Skimmilk

Subsequent to the H_2O_2-catalase treatment, Standard Plate Counts (SPC) were obtained on each concentrated skimmilk sample in order to determine the efficiency of each treatment. Total bacterial counts on all treated samples were relatively low. The hydrogen peroxide-catalase treatment as an adjunct to normal batch heat pasteurization proved to be quite effective in reducing the number of organisms in the milk samples. Consequently, the likelihood of those microbial survivors influencing final plate counts on the spray powders was substantially reduced. Data in Table 4 show the bacterial counts obtained from the concentrated milk samples following treatment with H_2O_2 and catalase.

Effect of Spray Drying Conditions on Initial Microbial Populations in Skimmilk Powder

The effects of various spray drying temperatures on *E. coli*, *M. flavus* and *B. subtilis* were determined. Exit air temperatures of 200 F, 180 F and 160 F were used. In order to eliminate the possibility of microbial

Table 4.--Plate counts of concentrated milk after treatment with H_2O_2 and catalase.

Sample	Organism inoculated	After treatment
SPC		cells/gm
1	E. coli	300
2	M. flavus	290
3	B. subtilis	240

contamination or subsequent survivors from one temperature to another, the higher temperature of 200 F was employed initially, followed by reductions to 180 F and 160 F. Data in Table 5 show the bacterial counts obtained from each dried milk sample. As the temperature of spray drying increased, the rate of microbial destruction increased. At each exit air temperature B. subtilis proved to be much more resistant to spray drying than the other organisms except in two cases at 160 F with M. flavus. E. coli was more sensitive to the various spray drying temperatures and M. flavus remained intermediate. Log reductions at the highest drying temperature of 200 F ranged from 0.51 to 3.30 depending on the microorganism. Even at the highest drying temperature (200 F) relatively large numbers of B. subtilis survived spray drying with the level of inoculum used.

Table 5.--Effect of various temperatures used in spray drying skim-milk on destruction of *E. coli*, *B. subtilis* and *M. flavus* when milk contained 1 x 10^6 organisms/ml.

Culture	Air temperature Outlet °F	Air temperature Inlet °F	Number of survivors per gram powder	Log reduction of viable organisms (log condensed-log powder	Survivors %
			TRIAL I		
E. coli	200	320	6.7×10^2	3.17	0.067
	180	285	1.9×10^4	1.72	1.9
	160	265	5.4×10^4	1.27	5.4
B. subtilis	200	300	1.4×10^5	0.84	14
	180	290	5.3×10^5	0.28	53
	160	270	6.5×10^5	0.19	65
M. flavus	200	310	2.5×10^4	1.60	2.5
	180	288	7.6×10^4	1.12	7.6
	160	272	8.0×10^5	0.10	80
			TRIAL II		
E. coli	200	288	5.0×10^2	3.30	0.05
	180	285	5.1×10^3	2.29	0.51
	160	265	1.1×10^4	1.95	1.1
B. subtilis	200	325	3.1×10^5	0.51	31
	180	310	3.8×10^5	0.42	38
	160	295	5.7×10^5	0.24	57
M. flavus	200	275	3.1×10^4	1.51	3.1
	180	262	6.8×10^4	1.17	6.8
	160	240	7.5×10^5	0.13	75

Effect of Storage on Microbial Populations of Spray Dried Skimmilk

The effect of storage on the survival of E. coli, B. subtilis and M. flavus in spray dried skimmilk was determined by storing the powders at 25 C, in sterile glass jars. Investigations regarding microbial survival in milk powders have indicated that the number of microorganisms in powdered milk decreases during storage. The rate of microbial die-off in milk powder also accelerated as the storage temperature increased. However, no comparative studies were performed for this particular research.

Microbial populations declined during storage, however, the amount of decrease varied between organisms. E. coli tended to die-off rather rapidly and was not observed in large numbers in the powder after one month of storage. After four weeks of storage, the average number of survivors in samples which had been initially contaminated with E. coli had dropped to less than 2.0% of the microbial population following drying. Both B. subtilis and M. flavus survived storage much more readily than E. coli, particularly after lengthy storage time. Figures 1, 2 and 3 illustrate the rates of microbial die-off during storage.

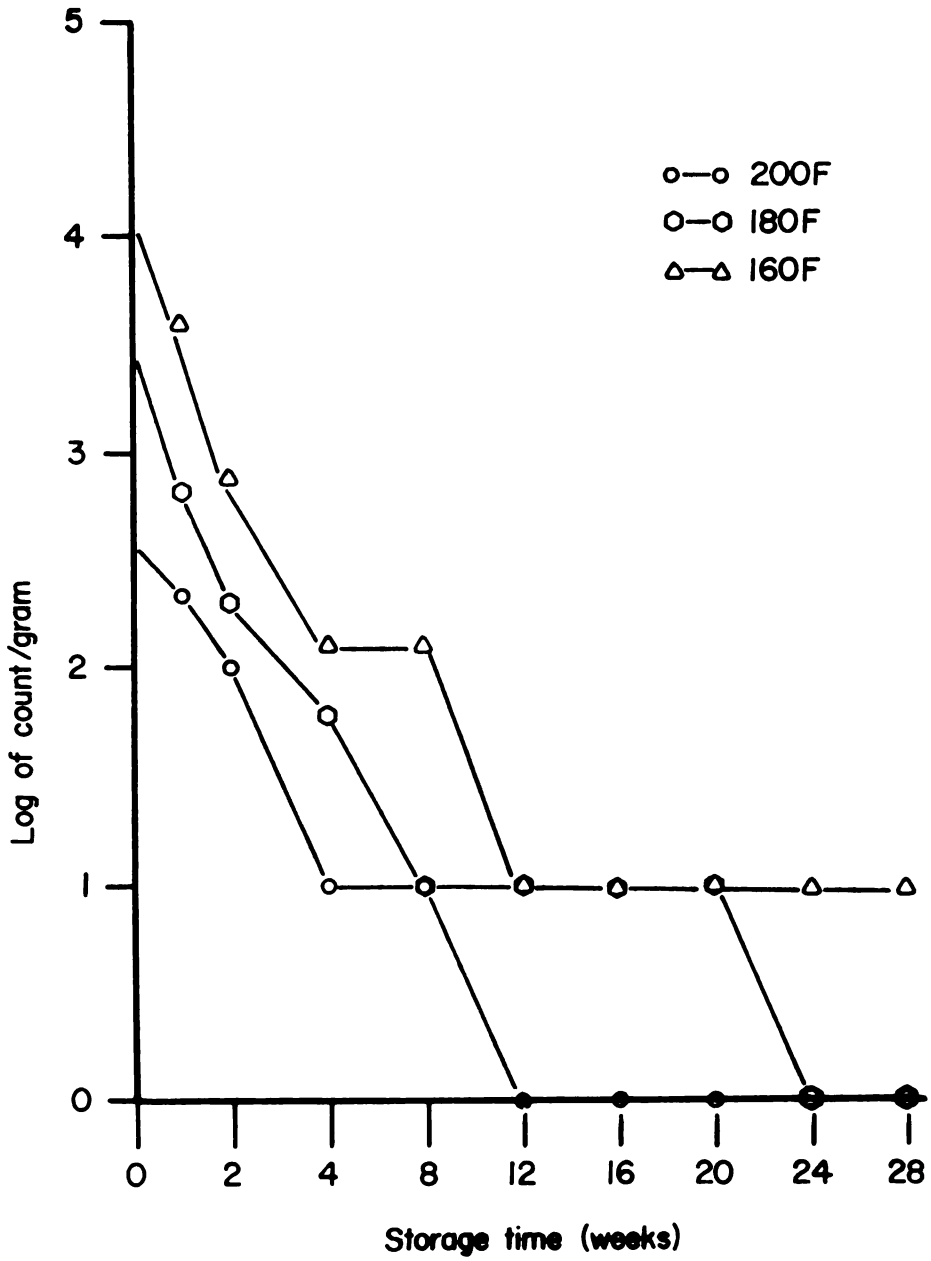


Fig. 1.--Survival of E. coli in spray dried skimmilk during storage at 25 C in milk dried with exit air temperatures of 160, 180 and 200 F.

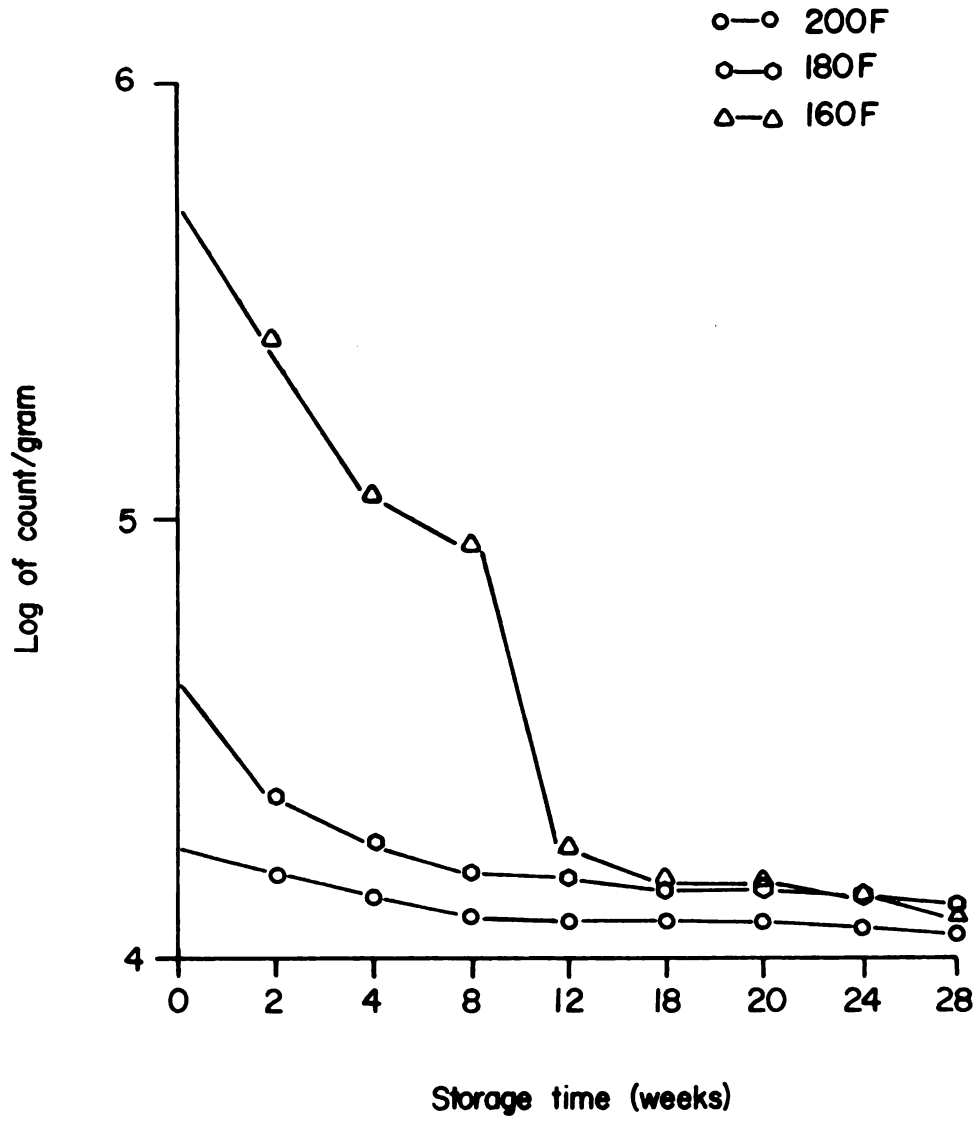


Fig. 2.--Survival of M. flavus in spray dried skimmilk during storage at 25 C in milk dried with exit air temperatures of 160, 180 and 200 F.

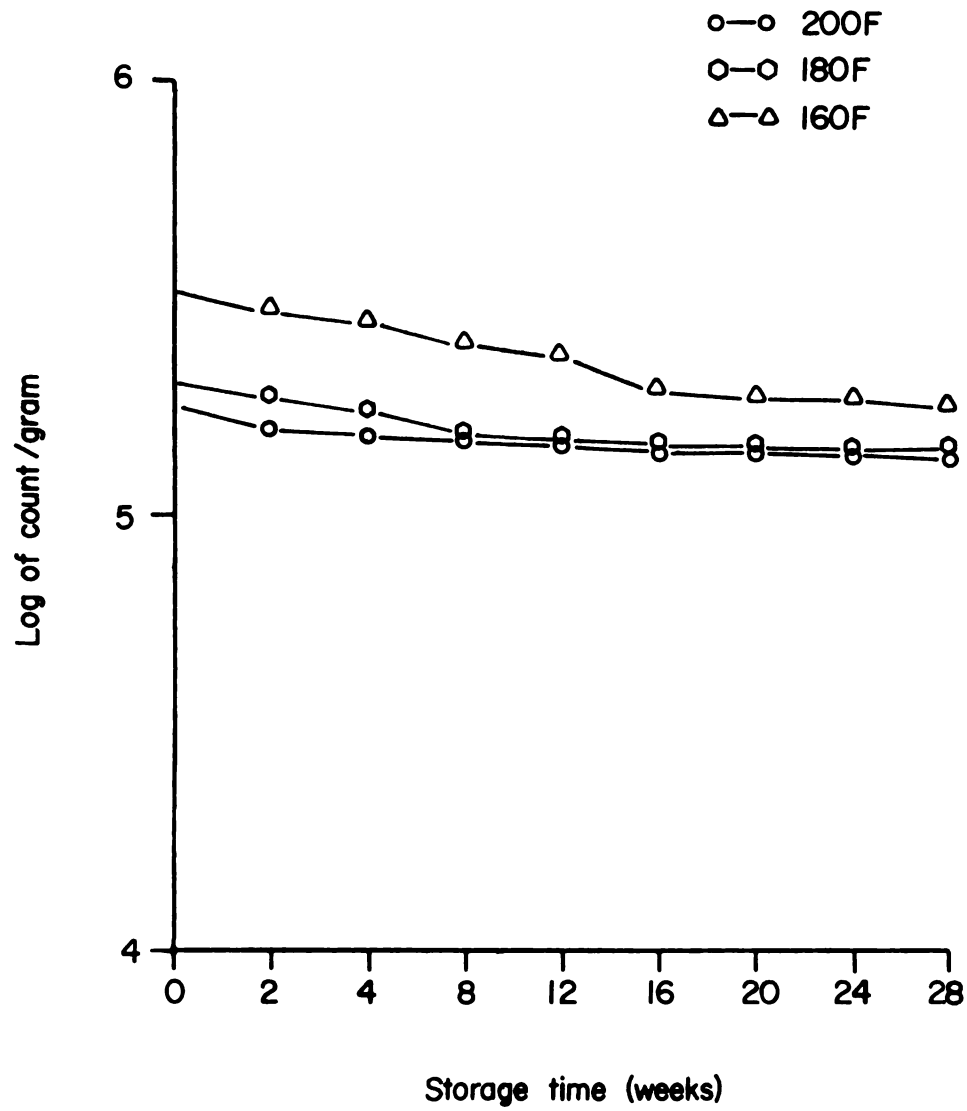


Fig. 3.--Survival of B. subtilis in spray dried skimmilk during storage at 25 C in milk dried with exit air temperatures of 160, 180 and 200 F.

Table 6.--Effect of storage on the number of E. coli in spray dried skimmilk powder.

Storage time (weeks) at 25 C	E. coli per gram of powder		
	Exit air temperature used		
	200 F	180 F	160 F
0	5.0×10^2	5.1×10^3	1.1×10^4
1	3.1×10^2	8.3×10^2	6.2×10^3
2	1.1×10^{2a}	3.7×10^2	9.0×10^2
4	1.0×10^{1a}	8.0×10^{1a}	2.0×10^2
8	1.0×10^{1a}	1.0×10^{1a}	2.0×10^{2a}
12	$<1.0 \times 10^{1b}$	1.0×10^{1a}	1.0×10^{1a}
16	$<1.0 \times 10^{1b}$	1.0×10^{1a}	1.0×10^{1a}
20	$<1.0 \times 10^{1b}$	1.0×10^{1a}	1.0×10^{1a}
24	$<1.0 \times 10^{1b}$	$<1.0 \times 10^{1b}$	1.0×10^{1a}
28	$<1.0 \times 10^{1b}$	$<1.0 \times 10^{1b}$	1.0×10^{1a}
32	$<1.0 \times 10^{1b}$	$<1.0 \times 10^{1b}$	1.0×10^{1a}

[a]Estimated count since all plates contained less than 30 colonies (3).

[b]Estimated count since the plates contained no colonies (3).

Table 7.--Effect of storage on the number of M. flavus in spray dried skimmilk powder.

Storage time (weeks) at 25 C	M. flavus per gram of powder		
	Exit air temperature used		
	200 F	180 F	160 F
0	3.1×10^4	6.8×10^4	7.5×10^5
2	2.7×10^4	4.3×10^4	4.8×10^5
4	2.2×10^4	3.4×10^4	1.5×10^5
8	1.9×10^4	2.8×10^4	9.5×10^4
12	1.8×10^4	2.6×10^4	3.2×10^4
18	1.8×10^4	2.4×10^4	2.7×10^4
20	1.8×10^4	2.4×10^4	2.5×10^4
24	1.7×10^4	2.3×10^4	2.3×10^4
28	1.5×10^4	2.0×10^4	1.9×10^4
32	1.5×10^4	2.0×10^4	1.9×10^4
36	1.4×10^4	2.0×10^4	1.9×10^4

Table 8.--Effect of storage on the number of B. subtilis in spray dried skimmilk powder.

Storage time (weeks) at 25 C	B. subtilis per gram of powder		
	Exit air temperature used		
	200 F	180 F	160 F
0	3.1×10^5	3.8×10^5	5.7×10^5
2	2.9×10^5	3.5×10^5	5.3×10^5
4	2.7×10^5	3.1×10^5	5.0×10^5
8	2.6×10^5	2.7×10^5	4.7×10^5
12	2.5×10^5	2.7×10^5	4.3×10^5
16	2.3×10^5	2.4×10^5	3.7×10^5
20	2.2×10^5	2.4×10^5	3.5×10^5
24	2.2×10^5	2.4×10^5	3.5×10^5
28	2.1×10^5	2.4×10^5	3.4×10^5
32	2.1×10^5	2.4×10^5	3.4×10^5

Effect of Spray Drying Temperature on the Moisture Content of Skimmilk Powder

Data in Table 9 shows the relationship between spray drying at various exit air temperatures and moisture in the final product. Each powdered milk sample was completely cooled before the moisture was determined. As expected the percent moisture decreased as the exit air temperature increased. The percent moisture ranged from 2.75% to 4.8% in powders dried at 200 F to 160 F, respectively. According to the specific grading requirements for nonfat dry milk (1), each sample may be classified as either extra grade (not greater than 4.0% moisture) or standard grade (not greater than 5.0% moisture).

Table 9.--Moisture content of spray dried skimmilks.[a]

Organism	Temperature (F) Exit air	Inlet air	% Moisture
E. coli	200	288	3.00
	180	285	4.60
	160	265	4.80
B. subtilis	200	325	3.06
	180	310	3.70
	160	295	4.70
M. flavus	200	275	2.75
	180	262	3.18
	160	240	4.68

[a]Moisture contents determined only on powders from experimental Trial II.

DISCUSSION

Effect of Spray Drying on Microbial Content of Skimmilk Powder

Spray drying under various operating conditions destroys microorganisms at different rates. However, under no circumstances has spray drying been shown to completely inactivate bacteria. Even when drying temperatures exceeded commercial drying operations, total microbial destruction was not observed.

Spray drying at all three exit air temperatures, 160, 180 and 200 F, destroyed numbers of E. coli, B. subtilis and M. flavus; however, none of these temperature exposures produced microbial free powder. In general, microbial survival decreased as exit air temperature increased from 160 to 200 F. The number of E. coli which survived each drying temperature was much lower in comparison with the levels of B. subtilis and M. flavus. The data in Table 5 indicates that at 200, 180 and 160 F, on an average of both trial runs, over 99, 98 and 96% of the original population of E. coli was destroyed, respectively. M. flavus showed an average percent destruction of 97, 93 and 22 at 200, 180 and 160 F. B. subtilis demonstrated

an average percent destruction of 77, 55 and 39 at these same temperatures. It can be concluded from these data, as well as other investigations, that E. coli in milk powder is not due to the heat stability of this organism but rather to plant equipment contamination by certain heat resistant strains. B. subtilis and M. flavus were more resistant to the drying conditions used, and B. subtilis was the most resistant organism at 200 F. The reduction of the number of B. subtilis during spray drying at each temperature was less than one log cycle. B. subtilis and M. flavus are examples of a spore former and a nonspore former, respectively, which exhibit relatively high resistance to adverse conditions such as high heat treatment during spray drying. This investigation confirms the work of earlier investigations which concluded that the number and type of survivors in the final spray dried powder depended primarily upon the nature of the liquid milk flora. Spore forming and nonspore forming thermodurics are more resistant to spray drying than organisms belonging to the family Enterobacteriaceae or the genus Staphylococcus.

Effect of Storage and Moisture Content on Survival of Microorganisms in Spray Dried Skimmilk

Higginbottom (28) suggested that there was a direct relationship between the moisture content of milk powder,

storage temperature and microbial survival. He concluded that a good milk powder should have a moisture content between 3% and 5%; moisture content higher than 5% favored microbial growth, especially in the presence of high relative humidity. Later Davis (14) demonstrated that the number of viable bacteria in milk powder decreased steadily unless the moisture content exceeded the acceptable level of 5%.

Investigations show that microbial populations decrease during extended periods of storage. In some of these studies microbial die-off during storage was described as a two-fold phenomenon where death occurred initially at a rapid rate followed by a relatively reduced rate. In other studies, microbial die-off was more varied. These data showed as much as 50 to 70% reductions after one month storage. On the other hand, other related results showed relatively small microbial die-off even after six months storage. From those observations the investigators concluded that microbial survival in powdered milk depended primarily upon the nature of the powder flora. Different microorganisms will exhibit different survival rates during storage. These variations in survival rates are related to differences in moisture content of the dried milk.

No two-fold die-off was observed with the microorganisms in this particular study. Figure 1 indicates

that there was a rapid decrease in the number of viable E. coli in each representative sample. After four weeks of storage the number of viable E. coli was extremely low, representing 2.0, 1.6, and 1.8% of the microbial population after drying at 200 F, 180 F, and 160 F, respectively. After 12 weeks of storage plate counts on the powder which had been spray dried at 200 F showed no viable E. coli, and after 24 weeks of storage plate counts on the powder spray dried at 180 F showed no viable E. coli. Plate counts on the powder spray dried at 160 F continued to show viable E. coli after 28 weeks of storage. Spore forming and most nonspore forming thermoduric organisms survive various storage conditions for long periods of time, whereas organisms such as E. coli and Salmonella die-off rather rapidly. B. subtilis and M. flavus were more resistant to storage than E. coli. Figures 2 and 3 sh ow that these organisms died at a gradual but slow rate.

Although variations of the moisture content of milk powder influence both growth and survival of microorganisms the decrease in viable counts exhibited by E. coli, M. flavus and B. subtilis cannot be exclusively associated with high versus low moisture contents since all the moisture levels were in an acceptable low range. The differences in moisture content between the powders spray dried at the three exit air temperatures were relatively small. The rates of microbial die-off can be

attributed to a combination of drying temperature, moisture content and susceptibility of the survivors to the low moisture levels. Since B. subtilis and M. flavus are able to survive adverse conditions of high heat and low moisture content these organisms were expected to demonstrate a larger number of survivors than E. coli even over an extended storage time.

Effect of Spray Drying on the Moisture Content of Skimmilk Powder

Spray drying has a main objective of removing moisture from liquid milk in order to form a powder (21). As liquid milk is atomized into the drying chamber, heated air is forced through the chamber. This heated air furnishes the heat necessary to evaporate moisture and it acts as a carrier for the moisture as it is removed from the drier. The design of the drying chamber, other equipment design and the desired moisture content directly influence the proper inlet and exit air temperatures.

The exit air temperature is the primary guide in controlling powder moisture, the higher the exit air temperature the lower the final powder moisture (10). During the spray drying operation, the final moisture can also be influenced by controlling product feed rate, varying residence time of droplets in the drying air, regulating the volume of hot air and controlling percent total solids of the concentrated milk (10, 21, 56). Spray

drying operators generally try to control the moisture content of the powdered milk by varying the exit air temperature. The main objective is to achieve an exit air temperature which will result in a product with a low moisture content without causing severe heat damage to the product.

The moisture content of spray dried milk is extremely critical because it acts as a major factor toward influencing the development of storage defects (10). Powders with high moisture content will not meet the extra grade or standard grade requirements for dry milks and will be quite susceptible to microbial and enzymatic alterations. Although there are economic disadvantages to spray drying at high exit air temperatures, it helps to preserve the powder considering the fact that microorganisms and enzymes need high moisture levels in order to be active.

The data in Table 9 demonstrates the influence exit air temperature has on final powder moisture. Increasing exit air temperature decreases the percent moisture of the powder. The moisture content of the skimmilk powders spray dried at 160 F and 200 F ranged from 4.80 to 2.75%, respectively.

SUMMARY AND CONCLUSIONS

This particular study was undertaken in order to obtain quantitative data concerning the survival of three nonpathogenic organisms which have previously been found in spray dried milk powder. Their incidence in powder and their ability to survive different spray drying temperatures has had limited exploration. A sufficient amount of broth culture either containing Escherichia coli, or Micrococcus flavus or Bacillus subtilis was inoculated into separate samples of concentrated skimmilk to obtain the desired concentration of 1×10^6 organisms per milliliter and spray dried at three different exit air temperatures, 160, 180 and 200 F. The number of organisms surviving these drying temperatures was determined by plating a representative sample of each dry powder on Violet Red Bile or Standard Plate Count Agar. In addition, survival of these organisms during storage at 25 C was determined over several months. It is recognized that spray drying at acceptable temperatures will destroy microorganisms; however, the extent of microbial destruction is much less than during roller drying. While spray drying at 160, 180 and 200 F destroyed numbers of E. coli, B. subtilis

and M. flavus, in no case was a microbial-free powder obtained with the amount of contamination used. B. subtilis, an aerobic spore former was the most heat resistant organism and M. flavus, a nonspore forming thermoduric organism, was the second most heat resistant organism. E. coli was the least heat resistant of the three organisms. These results correlate with findings of previous investigators who concluded that different microorganisms demonstrate varying survival rates under similar spray drying conditions.

During storage of the powders in sterile glass jars at 25 C for several months, E. coli was the least resistant, since the number of viable E. coli decreased quite rapidly. After four weeks of storage the viable count of E. coli in the powder which had been spray dried at 200 F was 2% of the population obtained immediately subsequent to drying. The powders which had been spray dried at 180 and 160 F showed a decrease in population of less than 1% after 8 and 12 weeks, respectively. After 9 months of storage the powders spray dried at 200, 180 and 160 F contained 45, 29, and 2.5%, respectively, of the M. flavus population subsequent to drying. After 8 months of storage the powders spray dried at 200, 180 and 160 F contained 68, 63 and 59%, respectively, of the B. subtilis population immediately after drying. None of the organisms demonstrated the two-fold die-off phenomenon

characterized by rapid initial reduction followed by a relative reduced rate, as some other investigations have shown.

The final moisture content of spray powder is quite critical in relationship to keeping quality because high moisture levels are responsible for both growth and survival of microorganisms in the milk powder during storage. The exit air temperature is used as a direct guide in controlling moisture levels of the final powder.

The moisture content of all powders dried in this study was within an acceptable low range, not greater than 5%. Consequently, during storage there was no danger of the numbers of these organisms increasing as long as the final moisture content was low and remained low. During storage the microbiological content of each powder decreased and the rates of decrease observed were due to a combination of drying temperatures, moisture content and the susceptibility of the survivors to the low moisture levels.

Since the use of nonfat dry milk and other spray dried milks in foods and in the manufacture of recombined products is increasing, identification of the microbial population in milk powders becomes more important. Investigations related to microbial survival during spray drying represents an interesting field which has had limited exploration.

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