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Survival of Selected Organisms During Spray Drying of Skim Milk and Storage of Nonfat Dry Milk[1]

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ABSTRACT

Pasteurized concentrated skim milk containing 35 to 40% total solids was inoculated with cultures of *Bacillus subtilis, Micrococcus flavus,* or *Escherichia coli* to contain 1×10^6 organisms per g and spray-dried to determine the effect of exit air temperatures of 93.3, 82.2, and 71.1 C on survival of the organisms and moisture content of the finished product. The numbers of survivors increased as the drying temperature decreased. The percent survivors varied from 27.57 in the product made from milk inoculated with *M. flavus* and dried at 71.1 C to 0.02 in the product made from milk inoculated with *E. coli* and dried at 93.3 C. The organism most resistant to drying and most persistent during storage was *B. subtilis,* followed by *M. flavus* and *E. coli,* with the latter showing low survival during drying and abrupt die-off during the first 4 weeks of storage. The moisture content of the dry milks varied from 2.75 to 4.80% with low moisture associated with high drying temperature.

In 1976, 426,000,000 kg of nonfat dry milk (NDM) was manufactured in the United States (*1*). Short shelf-life in fluid dairy products fortified with NDM has created an interest in the effect of spray drying and storage on viability of organisms commonly found in dry milks.

Investigations by Crossley and Mattick (*6*) showed that the microflora of spray-dried milk was influenced by the initial population in the milk, thermal treatment before and during drying, sanitation of equipment, and post-drying contamination. Crossley and Johnson (*5*) and Findlay et al. (*8*) emphasized the desirability of using the highest drying temperature possible without causing physical damage to the dry milk to accomplish maximum microbial destruction. A decrease in microbial content normally occurs during prolonged storage of dry milks, with the rate of die-off influenced by moisture content, storage temperature, and type of organisms present (*4,5,7,8,10*). Naguib et al. (*14*) identified the predominant organisms isolated from plate counts of dry milk as streptococci, micrococci, microbacteria, sarcina, and sporeforming bacilli. Foster et al. (*9*) and Keogh (*12*) stated the microbial content of dry milks is influenced more by type of organism than by number in the original milk, as spores and thermodurics tend to survive the drying process and are resistant to die-off during storage. Higginbottom (*11*) suggested a direct relationship existed between the moisture content of dry milk, storage temperature, and microbial survival, with good product containing between 3.0 and 5.0% moisture.

The investigation reported herein was conducted to determine the survival of *Bacillus subtilis, Micrococcus flavus,* and *Escherichia coli* inoculated into concentrated skim milk which was spray dried under various conditions. These organisms were selected to include a sporeformer, a vegetative cell resistant to heat and desiccation, and a thermally sensitive vegetative cell, all of which frequently occur in raw milk.

MATERIALS AND METHODS

Preparation of cultures

Stock cultures of *B. subtilis, M. flavus,* and *E. coli* were selected from the culture collection of the Department of Food Science and Human Nutrition at Michigan State University. *E. coli* was activated in nutrient broth at 37 C while *B. subtilis* and *M. flavus* were activated in Trypticase Soy Broth at 25 C. The cultures were transferred daily and incubated on a gyrotary shaker at 150 rpm for 3 consecutive days before inoculation into the concentrated milks.

Preparation of the concentrated milk

Fifty-gallon batches of whole milk from the Michigan State University Dairy Plant were separated into cream and skim milk. The skim milk was pasteurized at 62.8 C and concentrated in a vacuum pan to a ratio of approximately 4.3 to 1 or about 10 gallons containing 35 to 40% total solids. Since it was desired to reduce the natural bacterial content as much as possible before inoculation, without altering the physical condition of the milk, a hydrogen peroxide-catalase treatment (*15,17*) was used as an adjunct to pasteurization. The concentrated milk was warmed to 48.9 C and sufficient H_2O_2 to give a 0.05% concentration was added for a contact time of 20 to 30 min. The milk was then cooled to 37.7 C and an excess of catalase was added to decompose the H_2O_2. The KI test (*17*) for presence of H_2O_2 was repeated on the milk at 5-min intervals until complete dissipation of H_2O_2 was assured. The 10-gallon batches of concentrated milk were then inoculated with 24-h-old cultures of *E. coli, B. subtilis,* or *M. flavus* to provide approximately 1×10^6 organisms/g of milk. The *B. subtilis* cultures showed approximately 10 to 20% sporulation when inoculated.

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Spray drying

The milk was dried on a small commercial vertical down-draft, direct gas fired stainless steel spray dryer with a 55-ft tower manufactured by the Marriot Walker Corporation in Detroit, Michigan. The milk was introduced at 38 C with a high pressure pump through a high pressure spray nozzle. Each batch of condensed milk was divided into three portions which were dried at exit air temperatures of 93.3, 82.2, and 71.1 C. The operating conditions are reported in Table 1. To minimize the possibility of contamination or the adventitious effect of temperature, the first batch was always dried at 93.3 C, followed by 82.2, and 71.1 C.

Sampling and determining moisture

Representative samples of each batch of dry milk were collected in sterile glass jars and sealed to avoid change in moisture content. The amount of moisture in each sample was determined by the Karl Fischer titration using a Beckman model KF2 aquameter equipped with duo-platinum electrode. Sodium tartrate dihydrate containing 15.66% water was used as the standard for determining the titer of the Fischer reagent (*16*).

Microbiological analyses

All dry milk samples were analyzed according to procedures described in *Standard Methods for the Examination of Dairy Products* (*3*). Small glass beads in the phosphate buffered dilution blanks facilitated solubilizing the dry milk. Within 2 to 4 h after each batch was made, representative samples were plated to determine initial populations. Dry milk samples made from milk inoculated with *E. coli* were plated on Violet Red Bile Agar with an agar overlay and incubated at 35 C for 24 h. Samples inoculated with *M. flavus* or *B. subtilis* were plated on Standard Methods Agar (SMA) and incubated at 32 C for 48 h. Samples of all batches of product were stored in sterile glass jars at 25 C and the bacterial population determined at frequent intervals for 32 to 36 weeks.

RESULTS AND DISCUSSION

The total aerobic plate counts on typical batches of pasteurized concentrated milk after treatment with hydrogen peroxide and catalase were approximately 300, 290, and 240/g in the milks subsequently inoculated with *E. coli, M. flavus,* and *B. subtilis,* respectively. The above numbers of residual organisms had little effect on the population in the finished product since the inocula were approximately 1×10^6/g of concentrated milk.

Effect of spray-drying temperature on the bacterial content of the NDM

Data in Table 2 show the effect of spray-drying on bacterial content of the dry milks. The concentration ratio between the condensed milk and the NDM was determined by dividing the percent solids in the NDM by the percent solids in the concentrated milk. The population expected in the dry milk, if no destruction occurred, was calculated by multiplying the population in the condensed milk by the concentration ratio. The percent of organisms surviving spray drying was then computed by dividing the number of actual survivors by the number expected if no destruction occurred.

In all batches of NDM the percentage of survivors increased as the exit air temperature in the dryer decreased from 93.3 to 71.1 C (Table 2). The spore-forming *B. subtilis* was much more resistant to destruction during drying at 93.3 and 82.2 C than the non

TABLE 1. *Physical conditions used to prepare nonfat dry milk from concentrated milk inoculated with organisms and dried at temperatures as indicated. (Spray nozzle type SX, insert 65 and core 17A.)*

Physical condition	Concentrated milk with 37% T. S. inoculated with *B. subtilis*			Concentrated milk with 35% T. S. inoculated with *M. flavus*			Concentrated milk with 40% T. S. inoculated with *E. coli*		
Exit air (C)	93.3	82.2	71.7	93.3	82.2	71.1	93.3	82.2	71.1
Inlet air (C)	162.7	154.9	146.1	135.0	127.8	115.6	142.2	140.5	129.8
Ambient air (C)	23.9	23.9	23.9	31.1	31.1	31.1	23.9	23.9	23.9
Atomizing pressure (PSI)	1000	2100	2550	1200	2300	2600	1150	2300	3000
Pump vari-drive setting	0.9	3.2	3.5	1.8	3.2	3.3	1.0	3.0	3.6
Gas pressure (PSI)	4.4	4.6	4.4	5.0	3.0	3.9	4.0	4.0	3.5
ASME nozzle (inches)	1.3	1.3	1.3	1.3	1.3	1.3	1.5	1.5	1.5
Moisture in finished NDM (2)	3.06	3.70	4.70	2.75	3.18	4.68	3.00	4.60	4.80

TABLE 2. *Effect of various temperatures used in spray-drying concentrated skim milk on destruction of* B. subtilis, M. flavus *and* E. coli *when the concentrated milk was inoculated with* 1×10^6 *organisms/g.*

Culture used to inoculate	Air temp (C) Exit	Air temp (C) Inlet	% T.S. in conc. milk	% Moisture in NDM	Concentration ratio	Population expected in NDM if no destruction	Number of survivors in the NDM	% Survivors
B. subtilis	93.3	162.7	37	3.06	2.62	2.62×10^6	3.1×10^5	11.83
	82.2	154.5	37	3.70	2.60	2.60×10^6	3.8×10^5	14.62
	71.1	146.1	37	4.70	2.57	2.57×10^6	$5.7\ ^7\ 10^5$	22.18
M. flavus	93.3	135.0	35	2.75	2.77	2.77×10^6	3.1×10^4	1.12
	82.2	127.8	35	3.18	2.76	2.76×10^6	6.8×10^4	2.46
	71.1	115.6	35	4.68	2.72	2.72×10^6	7.5×10^5	27.57
E. coli	93.3	142.2	40	3.00	2.42	2.42×10^6	5.0×10^2	0.02
	82.2	140.5	40	4.60	2.38	2.38×10^6	5.1×10^3	0.21
	71.1	129.5	40	4.80	2.38	2.38×10^6	1.1×10^4	0.46

spore-forming organisms, but at 71.1 C the thermoduric *M. flavus* survived in greater numbers than did *B. subtilis*. *E. coli* was quite sensitive to the thermal exposure encountered during drying with destruction in excess of 99.54% in all cases. Another corresponding series of batches of dry milks (data not included) produced results which were markedly similar in all categories to the data in Table 2. *B. subtilis* and *M. flavus* are examples of a spore-former and none spore-former, respectively, which are common in raw milk and possess substantial resistance to the thermal process used during spray drying, as indicated by survival percentages of 22.18 and 27.57. These results substantiate previous reports (*9,12*) that the microbial content of dry milk is influenced by the type of organisms in the original milk. The substantial increase in survivors of *M. flavus* at 71.1 C compared to 82.2 C is attributed to the fact that *M. flavus* resists thermal destruction at temperatures up to about 80 C. Also, at 71.1 C, *M. flavus* has much more thermal resistance than *E. coli* and the vegetative cells of *B. subtilis*, as indicated by the data in Table 2. The inocula used in this experiment are greater than populations normally encountered in good quality concentrated milk but the numbers are reasonable for milk which has been carelessly handled. Admittedly, random contamination would not involve this magnitude of populations of pure cultures.

Bacterial standards published by the American Dry Milk Institute (*2*) permit maximum total aerobic plate counts of 50,000/g for Extra grade and 100,000/g for Standard grade NDM. The maximum permissible coliform count is 10/g. Moisture limitations are 4% for Extra and 5% for Standard grade. The only batch of NDM produced in this experiment which would comply with Extra grade standards is the one inoculated with *M. flavus* and dried at 93.3 C (Table 2). Obviously it is desirable to operate dryers at temperatures as high as consistent with good physical characterisitics in the finished dry milk product. It is also apparent that when milk with high initial populations of thermally resistant organisms is dried there may be sufficient survivors to represent a potential quality hazard when the product is reconstituted and used in food products.

Effect of moisture content and storage on the bacterial content of spray-dried NDM.

In all batches of dry milk produced in this experiment the percentage of die-off during storage at 25 C for 32 or 36 weeks increased as the number of organisms initially present increased and as the exit air temperature during drying decreased, with the amount of die-off varying amoung organisms (Table 3). *E. coli* decreased abruptly during the first 4 weeks while *B. subtilis* and *M.* flavus decreased gradually. When dry milks manufactured at exit air temperatures of 93.3 and 71.1 C are compared, the numbers of *B. subtilis*, *M. flavus*, and *E. coli* surviving the drying operation were 1.82, 22, and 25 times greater, respectively, at 71.1 than at 93.3 C. These results agree with a report by Keogh (*13*) indicating that the heat treatment the milk receives during drying does not eliminate all bacteria and most organisms present in dry milk are spore-formers or thermally resistant vegetative types such as micrococci or microbacteria. Also, Crossley (*4*) showed decreases of 50% in bacteria in dry milk stored 1 month in some instances, whereas in other instances there was little reduction in 6 months, thus leading to the conclusion that die-off depended on the nature of the organisms.

Data in Table 3 show the relationship between spray-drying at various exit air temperatures and moisture in the final product. The percent moisture varied from 2.75 to 4.80 with low moisture associated with high drying temperatures. All dry milk manufactured at an exit air temperature of 93.3 C and two of the three

TABLE 3. *Bacterial content of skim milk spray-dried at 93.3, 82.2 and 71.1 C during storage at 25 C for 32 to 36 weeks.*

Exit air temp (C)	93.3	82.2	71.1	93.3	82.2	71.1	93.3	82.2	71.1
% moisture	3.06	3.70	4.70	2.75	3.18	4.68	3.00	4.60	4.80
Storage time in wks at 25 C	inoculated with *B. subtilis*			inoculated with *M. flavus*			inoculated with *E. coli*		
	total count/g								
0	310,000	380,000	570,000	31,000	68,000	750,000	500	5,100	11,000
1	—	—	—	—	—	—	310	830	6,200
2	290,000	350,000	530,000	27,000	43,000	480,000	110	370	900
4	270,000	810,000	500,000	22,000	34,000	150,000	10	80	200
(% die-off after 4 wks)	(12.9)	(18.4)	(12.3)	(29.0)	(50.0)	(80.0)	(98.0)	(98.4)	(98.2)
8	260,000	270,000	470,000	19,000	28,000	95,000	10	10	200
12	250,000	270,000	430,000	18,000	26,000	32,000	10	10	10
16	230,000	240,000	370,000	—	—	—	1	10	10
(% die-off after 12 or 16 wks)	(25.8)	(36.8)	(35.1)	(41.9)	(61.8)	(95.7)	(99.8+)	(99.8+)	(99.9+)
18	—	—	—	18,000	24,000	27,000	—	—	—
20	220,000	240,000	350,000	18,000	24,000	25,000	1	10	10
24	220,000	240,000	350,000	17,000	23,000	23,000	1	1	10
28	210,000	240,000	340,000	15,000	20,000	19,000	1	1	10
32	210,000	240,000	340,000	15,000	20,000	19,000	1	1	1
36	—	—	—	14,000	20,000	19,000	—	—	—
(% die-off after 32 or 36 wks)	(32.3)	(36.8)	(40.4)	(54.8)	(70.6)	(97.5)	(99.8+)	(99.98+)	(99.99+)

manufactured at 82.2 C were within the moisture qualification for Extra grade (maximum 4.0%). All NDM manufactured at an exit air temperature of 71.1 C contained between 4.0 and 5.0% moisture, thus meeting the requirement for Standard grade. The fact that the percentage of bacterial die-off during storage increased as the moisture content of the product increased should not be misconstrued. The decrease is more intimately related to higher initial counts in the product dried at the lower temperature and the higher moisture is the result of the lower drying temperatures. Other workers have shown that when the moisture content is above 5.0%, survival during storage is favored by high moisture (*7,11*) but several reports (*7,10,11*) show the number of viable bacteria decrease consistently during storage of dry milk containing less than 5.0% moisture with the rate of die-off particularly dependent upon the nature of the microbial flora.

The exit air temperature is the primary determinant in controlling the moisture content of dry milk, with other factors being rate of injection, residence time of droplets in the chamber, volume of hot air, and percent solids in the concentrated milk. There are economic disadvantages to drying at high temperatures but the objective is to achieve an exit air temperature which will produce low moisture without physical damage to the finished dry milk product. NDM with moisture in excess of 5.0% has a predisposition to microbial spoilage and chemical deterioration by enzymatic and non-enzymatic reactions (*7,9,11,13*), therefore, operational conditions conducive to low moisture are desirable.

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