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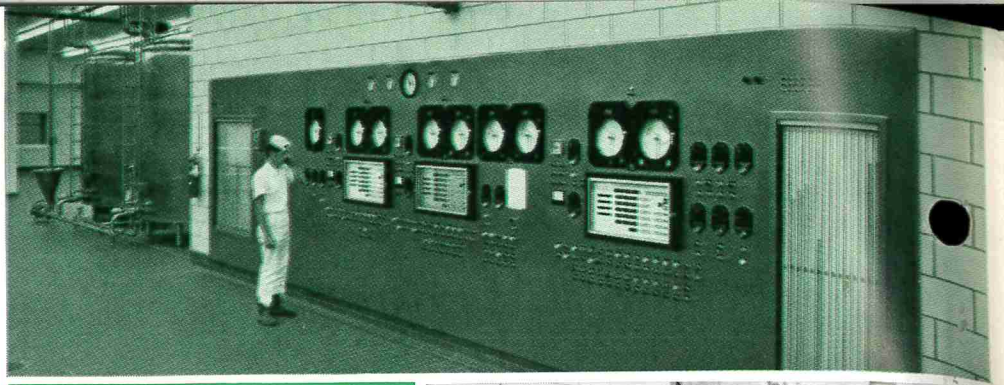
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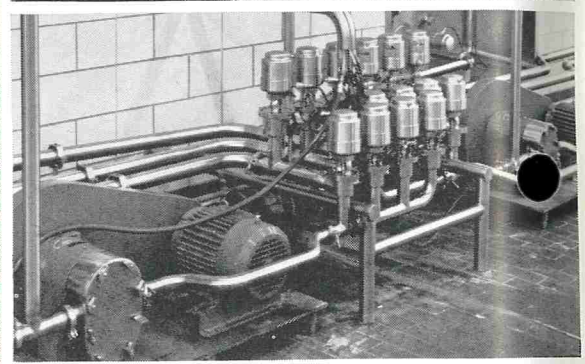
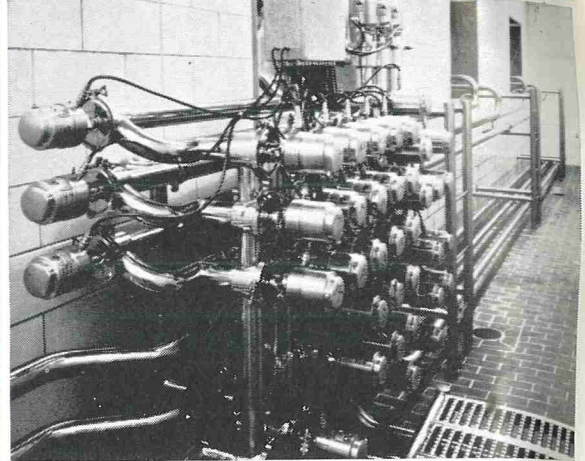
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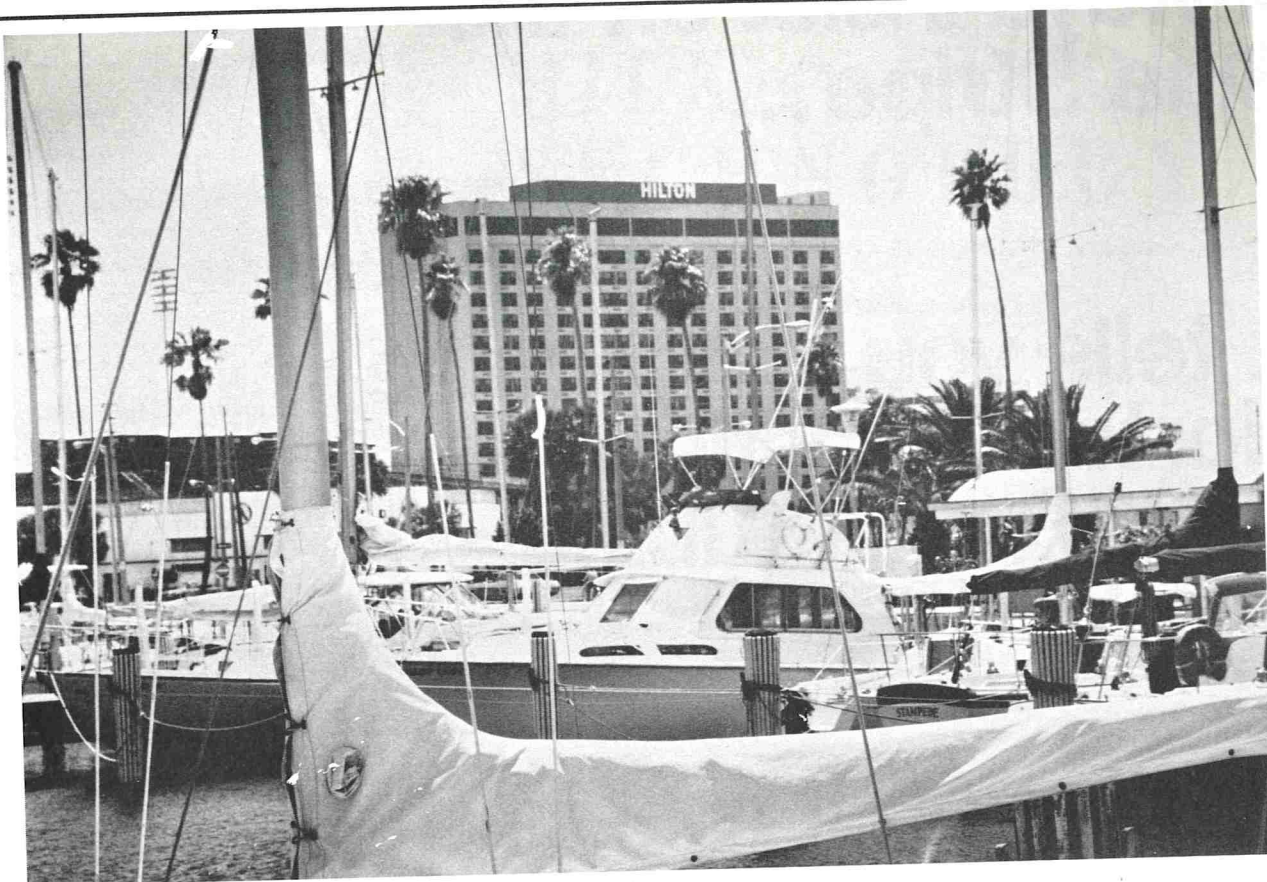
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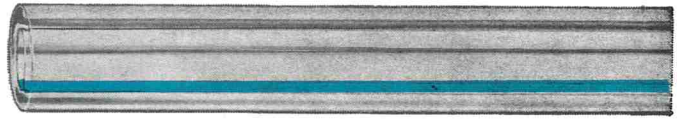
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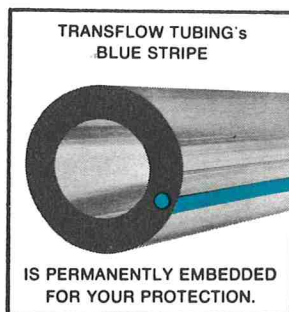
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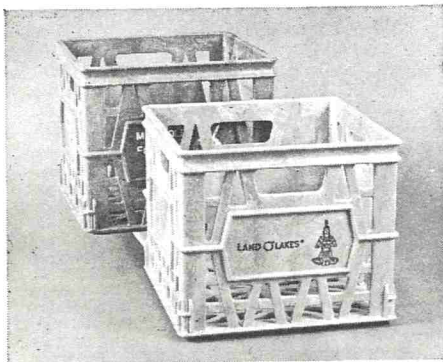
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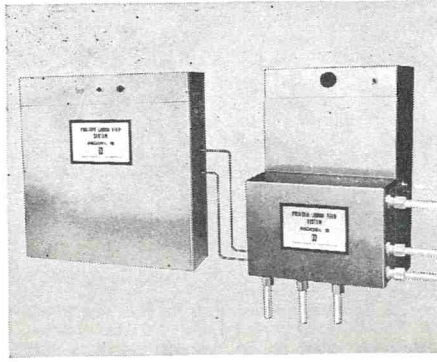
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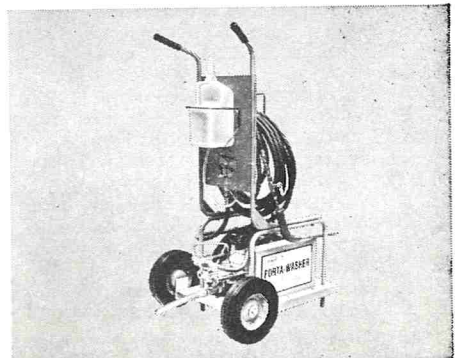
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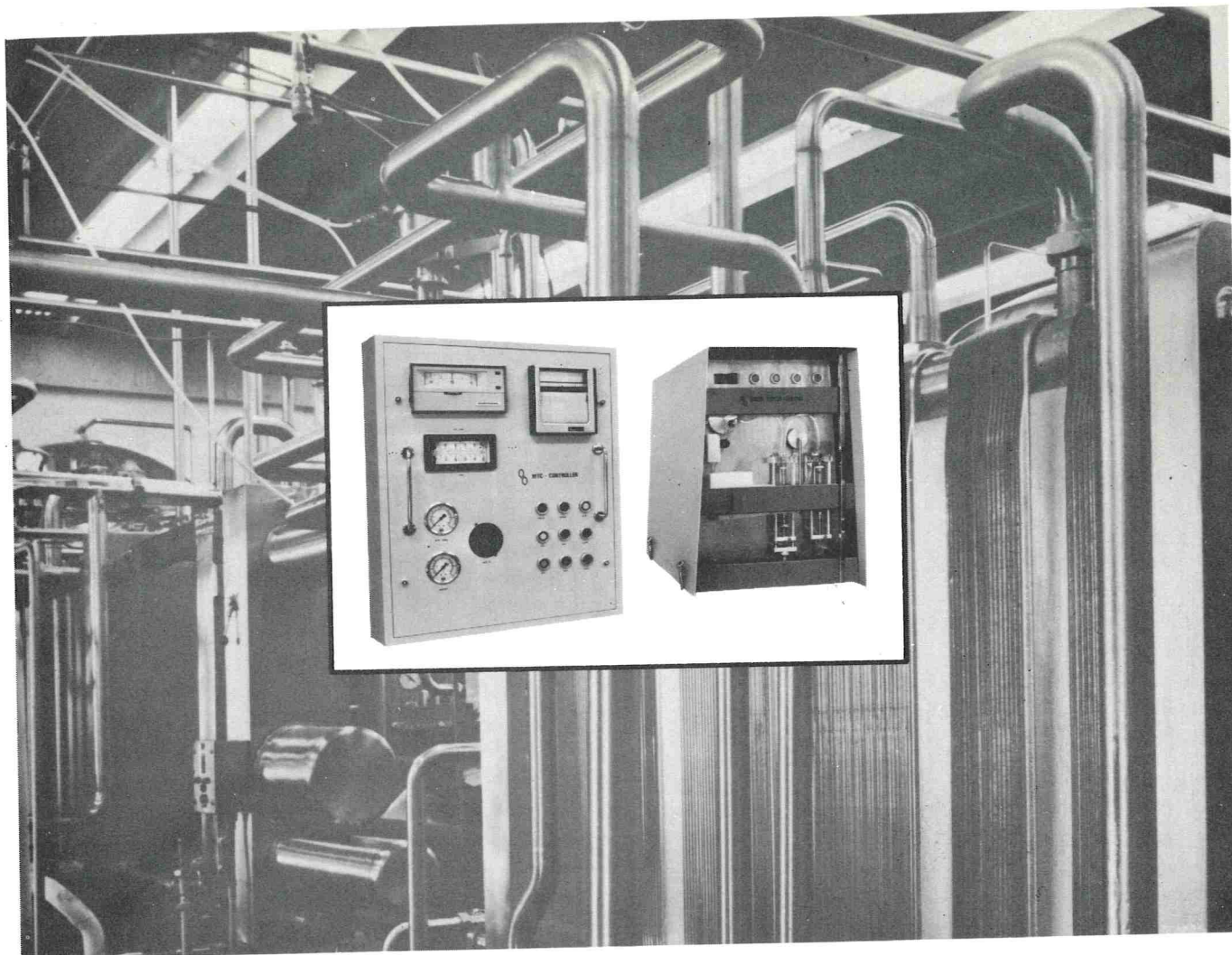
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LACTIC ACID FERMENTATION OF SOYBEAN MILK

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(Received for publication September 17, 1973)

ABSTRACT

Growth rates of 8 *Lactobacillus acidophilus* strains and four *Lactobacillus bulgaricus* strains were compared in soybean milk and soybean milk enriched with glucose, lactose, and sucrose. Four *L. acidophilus* strains grew well in soybean milk; the remainder grew better in soybean milk supplemented with glucose or lactose. In general, soybean milk was not an adequate media for strains of *L. bulgaricus*. Almost all these cultures, however, could adapt themselves to the environments of the media tested. A soybean milk drink fermented by *L. acidophilus* NRRL B-1910 was prepared and evaluated by a taste panel. The drink had a refreshing sweet-sour taste, and the beany flavor of soybeans was masked by the fermentation process.

Soybeans have been an important dietary item in the Orient for centuries. During this time, a large variety of food preparations have been developed from whole soybeans or from the water extract of wet ground beans. The water extract, known as soybean milk to westerners, is a beverage as well as a source of other foodstuffs, such as tofu. Although soybean milk is a popular beverage in some parts of Asia, people who are accustomed to the taste of cow's milk prefer it over soybean milk. Since fermentation often improves or modifies flavor, taste, and texture of soybeans (5), attempts have been made to develop fermented products from soybean milk by using cultures and methods employed in fermenting cow's milk. However, no acceptable product has resulted from soybean milk alone by conventional cheesemaking or yogurt processes.

Only by incorporating rennet extract and skim milk into soybean milk could Hang and Jackson (2, 3) prepare a satisfactory cheeselike product using *Streptococcus thermophilus* as the fermenting organism. Obara (6) suggested that an acceptable cheeselike product could be produced from soybean milk using a mixture of *Streptococcus cremoris* and *Streptococcus lactis*, provided the soy protein was first treated with appropriate proteolytic enzymes. Yamanaka et al. (8) developed a sour milk beverage, or yogurt, from an aqueous dispersion of skim milk solids, soy protein, and amino acids fermented by *Lactobacillus bulgaricus* and *S. thermophilus*. They claimed that adding amino acids to the fermented medium mask-

ed the characteristic flavor of soy protein.

Although the characteristics of fermented products are not always predictable and are affected by many factors, use of the proper microorganisms, perhaps, is paramount. Our objective was to determine whether various strains of *Lactobacillus acidophilus* and *L. bulgaricus*, two species used to produce acid-fermented cow's milk, could ferment soybean milk to make an acceptable product.

MATERIALS AND METHODS

Cultures

Cultures, including 8 strains of *L. acidophilus* and 4 strains of *L. bulgaricus*, were supplied by the ARS Culture Collection maintained at the Northern Laboratory. All lyophilized cultures were recultivated in deep liver medium (4) consisting of liver extract, 10% (v/v); yeast extract, 0.5% (w/v); tryptone, 1.0% (w/v); K₂HPO₄, 0.2% (w/v); glucose, 0.5% (w/v); and a few particles of liver.

Preparation of soybean milk

Hawkeye soybeans were soaked overnight at room temperature before they were washed and dehulled. Dehulled beans were blended for 2 min in a Waring Blendor with excess water, and the final volume of the blended mass was brought up to 10 times that of the weight of dry soybeans used. After the slurry was heated to boiling and filtered through double-layered cheesecloth, a milky-looking filtrate, known as soybean milk, was obtained.

Comparative growth of lactic acid bacteria

To prepare soybean milk medium, tubes containing 8 ml of soybean milk were autoclaved at 120 C for 15 min. After cooling, 2 ml of 20% (w/v) sterile sugar solutions (glucose, sucrose, or lactose) were aseptically added to each tube. The final concentration of sugar was 4% (w/v). Cultures (0.2 ml) were transferred directly from deep liver medium to the experimental media and incubated at 37 C. Preliminary work had indicated that 37 C was the optimal temperature at which fermented products having desirable texture and odor were obtained. Growth of each culture was judged by acid production, which was estimated by measuring the pH of the media. Incubation time was determined by appearance of coagulation in one of the media tested.

Yogurtlike products from soybean milk

Soybean milk-sugar media were prepared as described. Inoculum was prepared by first transferring the culture from deep liver medium to the experimental medium. Each culture was then serially transferred in the same medium at least twice, or until the medium had coagulated after 24 h of incubation. The inoculum so prepared was used at a level of 2.5% (v/v). The inoculated soybean milk was mixed thoroughly and incubated at 37 C.

Odor and flavor of fermented soybean milk

Soybean milk enriched with 4% sucrose was prepared and

¹Mrs. Lavanaya Kraidej was a trainee from Thailand supported by the Agency for International Development. Present address: Institute of Food Research and Product Development, P.O. Box 4-170, Bangkok 4, Thailand.

TABLE 1. pH OF THE GROWTH MEDIUM OF *Lactobacillus acidophilus* AND *L. bulgaricus* IN SOYBEAN MILK

Organism	NRRL No.	Incubation time (h)	pH of the growth medium			
			Soybean milk	Soybean milk with added		
				Glucose ¹	Lactose	Sucrose
<i>L. acidophilus</i>	B-2178	17	5.0	4.8	5.0	5.2
	B-2092	24	4.6	4.8	4.8	5.1
	B-1910	48	4.3	4.3	4.2	4.2
	B-1833	48	4.3	4.5	4.2	4.3
	B-629	17	6.2	4.7	4.7	5.8
	B-1911	24	6.1	4.5	5.5	5.8
	B-1912	48	5.9	4.2	4.7	5.8
	B-1858	48	6.6	6.6	6.6	6.6
<i>L. bulgaricus</i>	B-548	24	6.6	3.9	4.2	6.4
	B-734	48	6.7	6.7	6.7	6.7
	B-1909	48	6.5	6.6	6.7	6.6
	B-1918	48	6.6	6.5	6.5	6.6

¹Each carbohydrate added to give 4% (w/v) final concentration.

inoculated as before. *Lactobacillus acidophilus* NRRL B-1910 was the organism, and incubation was for 16 h. The fermented product was chilled and blended with an equal amount of cold water. Sucrose-enriched soybean milk used as control was treated similarly except that the inoculation step was omitted. All samples then were evaluated for odor and flavor by 14 members of the soy taste panel of the Northern Regional Research Laboratory. The members are specially trained to detect the different flavors occurring in soybeans.

RESULTS AND DISCUSSION

A typical set of the pH changes of soybean milk and soybean milk enriched with glucose, lactose, or sucrose for growth of *L. acidophilus* and *L. bulgaricus* is shown in Table 1. Among 8 strains of *L. acidophilus* studied, four of them—B-2178, B-2092, B-1833 and B-1910—grew almost equally well in soybean milk with or without addition of the several sugars. Evidently these strains utilize the carbohydrates in soybeans as their energy source. When these cultures were transferred serially 2 to 3 times in the same medium, their growth rate greatly increased, and they usually produced enough acid to coagulate the media (pH <5.2) after < 24 h of incubation.

Lactobacillus acidophilus, strain NRRL B-629, exhibited a high growth rate in soybean milk supplemented with glucose or lactose, but a slow rate in soybean milk alone or soybean milk enriched with sucrose. However, the growth rate of this strain increased by repeated transfer in the last two media. These results suggest possible preferential usage of glucose and lactose by this culture and its adaptability to sucrose utilization.

Soybean milk did not provide for good growth of *L. acidophilus* strains B-1911 and B-1912. Addition of glucose to soybean milk greatly increased growth of both strains, and added lactose also supported

growth but to a lesser extent; whereas, sucrose had no significant effect. Repeated transfer of these cultures in soybean milk did not significantly improve growth. Apparently, the carbon source(s) in soybean milk was the limiting factor for *L. acidophilus* strains B-1911 and B-1912, nor was lactose a favorable carbon source for these cultures. On the other hand, soybean milk enriched with glucose supported excellent growth.

Lactobacillus acidophilus strain B-1858 seemed to experience greater nutritional shock than did the other strains when transfers were made from stock cultures to experimental media. No change in pH was noted after 48 h of incubation in soybean milk or soybean milk enriched with sugars. Like other strains of *L. acidophilus*, this culture adapted to growth in soybean milk media after successive transfers, but in general, its growth rate was somewhat lower than that of the other strains.

All 4 strains of *L. bulgaricus* showed no growth in soybean milk after 48 h of incubation. Since addition of glucose or lactose to soybean milk stimulated growth of only *L. bulgaricus* strain B-548, the lack of readily metabolized sugars in soybean milk was the limiting growth factor for this culture. However, with successive transfers in soybean milk, *L. bulgaricus* strains B-548 and B-1909 adapted and grew well, whereas *L. bulgaricus* strains B-734 and B-1918 did not. Apparently, these media are not nutritionally adequate for growth of the last 2 strains.

Angeles and Marth (1) also found soybean milk to be a satisfactory growth medium for some lactic acid bacteria; e.g., *S. thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus pentosus*, *Leuconostoc mesenteroides*. It is indeed interesting to find that these species of bacteria grow well in soybean milk alone, since they are considered fastidious. Also, soybeans are low in readily metabolized sugars, the principal components of the soluble carbohydrates being stachyose, raffinose, and sucrose (7).

TABLE 2. FLAVOR AND ODOR EVALUATION OF *L. acidophilus* B-1910 FERMENTED SOYBEAN MILK AND CONTROL SOYBEAN MILK BY TASTE PANEL

Description	Sensory response of 14 tasters (%)	
	Control milk	Fermented milk
Odor		
Grassy/beany	36	7
Acid/sour	—	29
Raisins	14	57
Flavor		
Grassy/beany	50	7
Acid/sour	14	93
Sweet	64	29
Raisins	14	36
Astringent	21	29

Our data indicate that growth of the lactic acid bacteria we studied, and their ability to adapt themselves in different media, varied greatly among different strains of the same species as well as between the two species.

Fermented yogurtlike soybean milk products

After 2 or 3 transfers in soybean milk medium, all cultures—except *L. acidophilus* strains B-1911, B-1912 and B-1858, and *L. bulgaricus* strains B-734 and B-1918—grew well in soybean milk and formed an acid curd after 16 h of incubation at 37 C. The texture, acidity, and flavor of the curd made by each culture, however, varied considerably. From our experience, *L. acidophilus* strain B-1910 consistently produced a better product than the other strains.

The product from fermenting soybean milk with *L. acidophilus* strain B-1910 was eggshell (white) in color, had a sour but not harsh flavor, and had a smooth custardlike texture. When it was spooned out of its container, the appearance of free whey indicated the need for a stabilizer. This product, therefore, was not evaluated by the taste panel. When 4% sucrose was added to the soybean milk before fermentation, the final product had a pleasant sweetish-sour taste. Sucrose was added to the soybean milk not so much for growth of the organism, but to give a balance between acidity and sweetness of the product. The balanced sweetish-sour taste seems to be an important factor for acceptance of the product.

Taste panel evaluation of fermented soybean milk drink

When the yogurtlike soybean milk product made by *L. acidophilus* B-1910 was chilled and blended with an equal amount of cool water, a surprisingly refreshing sour drink with a raisin flavor resulted. The fermented soy drink was evaluated by 14 members of the soy flavor panel. Table 2 lists the flavor and odor descriptions of the control and fermented soybean milk drink as determined by the taste panel. Only 1 (7%) of the 14 tasters detected the presence of beany flavor and odor in the fermented product, whereas 50% of the panel described the control soybean milk drink as having a beany flavor. Although 36% and 57% of the panel characterized the fermented drink as having a raisin flavor and odor, respectively, only 14% gave the same description for the control drink. The origin of the raisin flavor and odor can-

not be accounted for. However, the description was appraised as accurate.

Our results, therefore, suggest that the fermentation process has suppressed the beany flavor of soybeans. Whether the beany flavor was merely masked by other flavors derived from fermentation, or modified somehow, was not determined.

Yogurt and acid milk beverages are widely consumed by different races. However, in many parts of the world cow's milk is not readily available or is prohibitively expensive. Fermented soybean milk as described might well serve as a substitute in these areas, and research and development leading to acceptable fermented soybean milk products offer much promise.

ACKNOWLEDGMENTS

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EFFECT OF STORAGE AT 5 C ON SURVIVAL OF *VIBRIO PARAHAEMOLYTICUS* IN PROCESSED MARYLAND OYSTERS (*CRASSOSTREA VIRGINICA*)¹

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ABSTRACT

Freshly processed Maryland oysters (*Crassostrea virginica*) were inoculated with various levels of *Vibrio parahaemolyticus* Strain 3525 (03:K30), Kanagawa negative, and Strain 8700 (04:K11), Kanagawa positive. Inoculated oysters were stored at 5 C for up to 13 days and numbers of viable cells determined at regular intervals by both a direct plating method and a most probable number (MPN) method. The number of cells detected was dependent on strain, inoculum level, and method of enumeration. In general, the direct plating method was unreliable and results varied according to plating medium used. At an inoculum level of 10⁶ cells/g, viable cells of Strain 3525 and Strain 8700 were not detected by the direct plating method after 3 and 5 days of storage, respectively, while by the MPN method low numbers of Strain 3525 and Strain 8700 were still detected after 7 and 13 days of storage, respectively. At inoculum levels of 10⁴ and 10² cells/g, the direct plating method did not accurately enumerate viable cells. Neither strain was detected by the MPN method following 5 days of storage at these inoculum levels. Loss of viability of both strains occurred most rapidly within the first 24 h and in some instances was as great as 3 log cycles. In general, higher levels of survivors of Strain 8700 than Strain 3525 were noted throughout the study. The pH change of oysters during storage was slight and could not account for the loss of viability of either strain.

Vibrio parahaemolyticus is the causative organism in approximately 50% of the food-borne illnesses in Japan where outbreaks occur chiefly due to consumption of raw fish. This microorganism has been demonstrated to be present in many coastal environments, including shellfish and waters of the Chesapeake Bay (10, 11). Oysters are often consumed raw, and therefore are a potential source of outbreaks of *V. parahaemolyticus* food poisoning. Baross and Liston (4) reported a range of 10² to 10⁵ cells/g (average, 5 × 10⁴ cells/g) in shucked oysters (*Crassostrea gigas*) harvested from Pacific northwest coastal waters. Thomson and Thatcher (15) found that 22% of the shellfish sampled on the Canadian Atlantic coast yielded *V. parahaemolyticus* in low levels. One-half of these samples required enrichment before the microorganism could be detected. Bartley and Slanetz (5) reported that oysters harvested from the coastal

waters of New Hampshire contained approximately 5 × 10² *V. parahaemolyticus* cells/g in September and that the levels decreased during October and November as the temperature of the water decreased.

Sensitivity to cold temperatures has been suggested as a reason for the seasonal variation in incidence and level of *V. parahaemolyticus* in shellfish and coastal waters (13). It has been stated in a recent study (9) that *V. parahaemolyticus* cannot grow and, in fact, perishes at temperatures below 6 to 8 C. However, results of this study indicated that Pacific oysters (*Crassostrea gigas*) which were contaminated with 10⁴ to 10⁵ streptomycin-resistant cells/g, frozen and then stored for 130 days, contained as many as 10³ to 10⁴ cells/g. Enumeration was done by surface plating on trypticase soy agar containing streptomycin. In addition oysters which were similarly contaminated and then refrigerated for 14 days, contained up to 10³ cells/g.

A confirmed outbreak of *V. parahaemolyticus* food poisoning due to consumption of processed Maryland oysters has not been reported to date. The purpose of this investigation was to follow the survival of *V. parahaemolyticus* at various levels in Maryland oysters during the recommended storage period at 5 C. In addition, currently recommended media and methods for enumeration of *V. parahaemolyticus* in oysters were compared.

MATERIALS AND METHODS

Cultures

Two strains of *V. parahaemolyticus* isolated from an outbreak of food poisoning in Maryland (August, 1971) were obtained through the courtesy of Dr. M. Fishbein, Division of Microbiology, Food and Drug Administration, Washington, D. C. Strain 3525 (serotype 03:K30), Kanagawa negative, was isolated from steamed crabs and Strain 8700 (serotype 04:K11), Kanagawa positive, was isolated from the stools of the patients. Both cultures were maintained on trypticase soy agar (BBL, BioQuest) containing 2.5% NaCl and a long-term-preservative-medium (LTPM) (3).

Preparation of bacterial inocula

Stock cultures were streaked onto thiosulfate-citrate-bile salts-sucrose agar (TCBS) (BBL, BioQuest) plates. Single colonies were used to inoculate tubes of trypticase soy broth (TSB) (BBL, BioQuest) containing 2.5% NaCl, followed by

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incubation at 32 C for 14 to 16 h. A 10.0% inoculum was then made into TSB containing 2.5% NaCl followed by incubation in a gently agitating water bath at 32 C. When the optical density at 620 nm was equal to 0.200 (Bausch and Lomb, Spectronic 20), the cultures were used to prepare inoculums.

Inoculation and storage of processed oysters

Freshly processed oysters (*C. virginica*), standard brand, were obtained at a local processing plant in Maryland and refrigerated at 5 C until the next day. Six 1000-g aliquots of processed oysters were distributed into commercial 1-gal oyster cans and inoculated so that concentrations of 10^6 , 10^4 and 10^2 cells/g of each strain were obtained. The cans were tightly closed, agitated, and then sampled immediately for zero hour data. Following sampling, oysters were stored at 5 C for up to 13 days. The recommended maximum storage period for processed oysters in Maryland at this temperature is 10 to 13 days.

Bacterial counts

The initial level of inoculated *V. parahaemolyticus* was determined by both a most probable numbers (MPN) method using glucose-salt-teepol-broth (GSTB) (2) and a direct plating method using 0.1-ml quantities of appropriate dilutions spread on plates of TCBS agar, Modified Twedt starch agar (MT) (17) and standard methods agar (SMA) (BBL, BioQuest) containing 1% NaCl. Subsequent sampling times were as follows: MPN at 1, 2, 3, 5, 7, 10, and 13 days; direct plating at 3, 6, 9, 12 h, and 1, 2, 3, 5, 7, 10, and 13 days. At each sampling interval 50 g of oysters were added to 450 ml of sterile 0.5% peptone-1% NaCl dilution medium and blended for 1 min. Subsequent dilutions were made and used to inoculate both MPN tubes and the direct plating medium. All dilution blanks used were 0.5% peptone-1% NaCl and were tempered to room temperature before use. All *V. parahaemolyticus*-like (VPL) colonies on TCBS (8), MT (17) and SMA 1% NaCl were counted. On SMA 1% NaCl, suspect VPL colonies were identified by comparison to stock cultures and confirmed as VPL by culturing on TCBS. Therefore counts reported represent high levels of inoculated *V. parahaemolyticus* plus low levels of oyster flora having similar colonial morphology.

pH

At each daily sampling interval, the pH of homogenized oysters was determined using a Beckman Expandomatic pH meter

RESULTS

Inoculation of oysters with 10^6 cell/g of Strain 3525

The level of cells enumerated immediately following inoculation of the oysters was dependent upon the plating medium used (Fig. 1). For example, on non-selective SMA 1% NaCl, 9.7×10^6 cells/g were enumerated initially while on the selective MT and TCBS media, 1.4×10^6 cells/g and 5.1×10^5 cells/g, respectively, were detected. The three-log cycle difference in counts obtained using SMA 1% NaCl and TCBS media is of interest. Exclusive use of TCBS medium would have resulted in an inaccurate estimate of the numbers of this strain of *V. parahaemolyticus* in the oysters. Counts on MT medium decreased sharply and after 24 h no cells

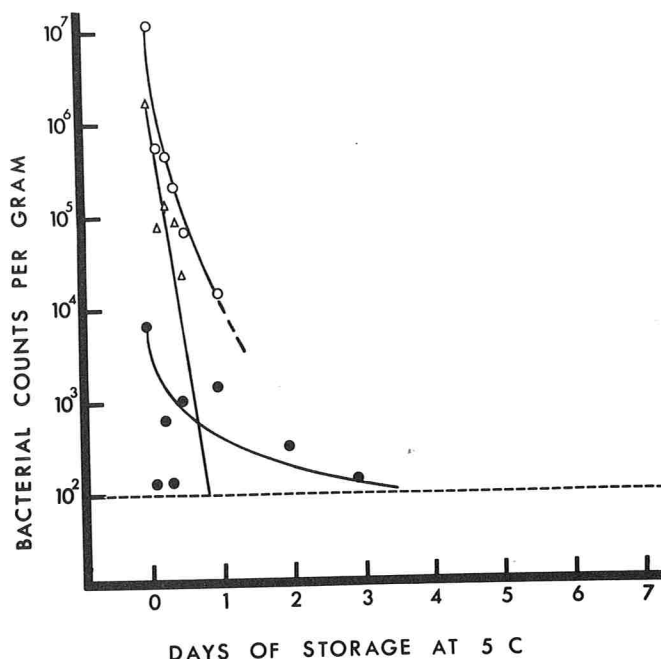


Figure 1. Bacterial counts following inoculation of processed oysters at 5 C with *Vibrio parahaemolyticus* Strain 3525, as enumerated on standard methods agar containing 1% NaCl (open circles), thiosulfate-citrate-bile salts-sucrose agar (closed circles), and Modified Twedt medium (open triangles). Counts represent all *V. parahaemolyticus*-like colonies (See Materials and Methods section).

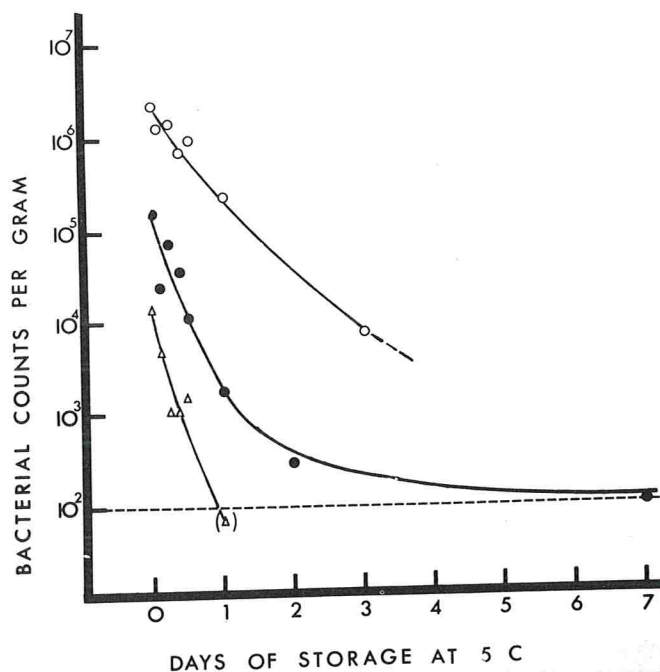


Figure 2. Bacterial counts following inoculation of processed oysters at 5 C with *Vibrio parahaemolyticus* Strain 8700, as enumerated on standard methods agar containing 1% NaCl (open circles), thiosulfate-citrate-bile salts-sucrose agar (closed circles), and Modified Twedt medium (open triangles). Counts represent all *V. parahaemolyticus*-like colonies (See Materials and Methods section).

TABLE 1. pH OF PROCESSED OYSTERS AND SURVIVAL OF *Vibrio parahaemolyticus* STRAIN 3525 IN PROCESSED OYSTERS STORED AT 5 C AS ENUMERATED BY THE MPN METHOD

Storage period (days)	pH	Inoculum level					
		10 ⁶ cells/g		10 ⁴ cells/g		10 ² cells/g	
		pH	MPN/g	pH	MPN/g	pH	MPN/g
0	6.4	>1.1 × 10 ⁶	6.4	24	6.4	9.3	
1	6.4	7.5 × 10 ²	6.6	0	6.6	0	
2	6.4	110	6.5	0.36	6.6	0	
3	6.4	24	6.6	0	6.4	0	
5	6.6	0.36	6.6	0	6.5	0	
7	6.2	9.3	6.4	0	6.5	0	
10	6.0	0	—	— ^a	—	—	

^aFurther counts not done if previous sample was zero.

were recovered on this medium even though the count on SMA 1% NaCl was still about 10⁴ cells/g. No VPL cells were detected after 3 days following inoculation with Strain 3525 regardless of the plating medium used.

Data obtained using the MPN method are presented in Table 1. Immediately following inoculation of the oysters with Strain 3525, 1.1 × 10⁶ cells/g were enumerated. This number decreased three log cycles within one day and only very low numbers of cells were detected after 7 days of storage.

Inoculation of oysters with 10⁶ cells/g of Strain 8700

The initial level of cells enumerated by direct plating was again dependent on type of medium used; however, in this instance higher numbers of VPL cells were obtained on TCBS medium than on MT medium (Fig. 2). Counts on TCBS medium at storage intervals of up to 3 days were one to two log cycles lower than counts on non-selective SMA 1% NaCl medium. Counts on MT medium were about three log cycles lower than those on the non-selective medium and no viable cells were detectable after 24 h when 0.1-ml portions of 10⁻¹ dilutions of oyster sample were plated on MT medium. MPN results obtained (Table 2) indicated that higher levels of survivors were present in oysters inoculated with Strain 8700 than in oysters inoculated with Strain 3525.

Inoculation of oysters with 10⁴ and 10² cells/g of *V. parahaemolyticus*

The direct plating method was inadequate for accurate estimation of viable cells during storage at 5 C at either inoculum level. No VPL colonies were seen on either MT or TCBS media following inoculation with Strain 3525. Only occasional VPL colonies were seen on TCBS medium following inoculation with Strain 8700. (Data not shown).

The MPN method yielded values (Tables 1 and 2) that were lower than the expected statistical range limits for these initial levels (1). Neither strain was

detected by the MPN method following 5 days storage at 5 C.

pH of oysters during storage at 5 C

The pH of the blended oysters was noted at each MPN sampling interval (Tables 1 and 2). It is unlikely that the small changes in pH during storage at 5 C could account for the loss of viability of the strains.

DISCUSSION

To date there has been no confirmed outbreak of *V. parahaemolyticus* food poisoning attributed to consumption of oysters. As shown by this study, high numbers of *V. parahaemolyticus* inoculated on the surface of processed oysters were reduced several log cycles within 24 h at 5 C. It is possible that *V. parahaemolyticus* localized in the intestinal tract of oysters might be afforded more protection. Covert and Woodburn (6) reported that fish homogenate tended to stabilize cells of *V. parahaemolyticus* at 48, 5, -5, and -18 C. Food poisoning resulting from consumption of the low numbers of *V. parahaemolyticus* normally found (4, 5, 15) in freshly harvested Maryland oysters properly stored at 5 C would appear unlikely. However, the possibility of the disease cannot be entirely dismissed since infective levels of the bacterium may develop if these shellfish are mishandled and held at higher temperatures particularly if the initial level of contamination is high. Moreover, raw oysters containing even low numbers of cells could serve as a source of contamination to other products.

Under conditions of storage that would permit abundant growth of *V. parahaemolyticus* in oysters, normal processes of spoilage would also probably occur. The latter is usually accompanied by a lowering of pH as well as an increase in growth of spoilage microorganisms (14). Since growth of *V. parahaemolyticus* in laboratory prepared media was observed to be optimal at pH 8 (16) any lowering of

TABLE 2. pH OF PROCESSED OYSTERS AND SURVIVAL OF *Vibrio parahaemolyticus* STRAIN 8700 IN PROCESSED OYSTERS STORED AT 5 C AS ENUMERATED BY THE MPN METHOD

Storage period (days)	pH	Inoculum level					
		10 ⁶ cells/g		10 ⁴ cells/g		10 ² cells/g	
		pH	MPN/g	pH	MPN/g	pH	MPN/g
0	6.5	>1.1 × 10 ⁶	6.5	>110	6.5	24	
1	6.5	4.6 × 10 ⁴	6.5	12	6.6	0	
2	6.5	4.6 × 10 ³	6.5	24	6.6	0	
3	6.5	>1.1 × 10 ³	6.6	23	6.6	0	
5	6.3	1.5 × 10 ³	6.4	0	6.6	0	
7	6.2	46	6.4	0	6.4	0	
10	6.1	—	—	— ^a	—	—	
13	5.9	24	—	—	—	—	

^aFurther counts not done if previous sample was zero.

TABLE 3. RECOVERY OF LOG-PHASE CELLS OF *Vibrio parahaemolyticus* GROWN IN TRYPTICASE SOY BROTH CONTAINING 2.5% SALT

Strain	Plating medium		
	SMA 1%	TCBS	MT
3525	1.8×10^{8a}	1.7×10^6	7.1×10^6
8700	6.7×10^7	5.8×10^7	1.0×10^7

^aCounts per ml.

the pH would adversely affect growth of *V. parahaemolyticus*.

The inability of either direct plating or the MPN method to recover VPL cells following inoculation of oysters at 5 C with 10^4 and 10^2 cells per gram of both strains was unexpected. This rapid loss of viability might be the result of the "cold-shock" phenomenon (7). Variations in media salt concentrations have also been shown to affect recoverability of *V. parahaemolyticus* (6). In addition, the possible presence of deleterious chemical constituents in oysters could be responsible for injuring the cells since it has been shown that oyster extracts have an inhibitory effect on other pathogenic microorganisms (12).

The above stresses also might explain the differences obtained with selective versus non-selective media during storage when the inoculum level was 10^6 cells/g. Counts on selective media were at certain storage intervals as much as three log cycles less than those obtained on non-selective medium. Counts were lower on MT and TCBS media than on SMA 1% NaCl medium when log-phase populations of *V. parahaemolyticus* grown in TSB containing 2.5% NaCl were plated (Table 3). These differences in recovery were more pronounced with Strain 3525 than with Strain 8700; however, in both instances, the differences were increased following exposure to processed oysters at 5 C (Fig. 1 and 2).

Survival of *V. parahaemolyticus* in processed Maryland oysters stored at 5 C was variable depending on strain and initial level of contamination. The two strains used in this study differed in that Strain 3525 was isolated from the suspect food and Strain 8700 was isolated from the stools of patients. It has been reported that the serotypes isolated from patients following *V. parahaemolyticus* food poisonings are not always the same serotypes that are isolated from the incriminated foods (8). In this study the Kanagawa positive Strain 8700 appeared

more resistant than Strain 3525 to loss of viability during storage in processed oysters at 5 C.

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EFFECT OF FREEZE DRYING, SPRAY DRYING, AND STABILIZERS ON FUNCTIONAL CHARACTERISTICS OF FISH PROTEIN CONCENTRATE

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ABSTRACT

A study was conducted to define some of the functional properties of IPA extracted hake FPC (fish protein concentrate). Also, we investigated possible methods of improving the functional properties of FPC by foaming a water slurry and adding stabilizers and then spray drying or freeze drying. Certain functional properties such as bulk density, pH, wettability, percent overrun, emulsion stability, emulsion capacity, water swelling index and water binding index, and suspended solids were measured. The results were compared with soy flour and soy isolate. Spray drying or freeze drying of an aqueous slurry of FPC brought about the following significant changes: (a) made a totally wettable material, (b) slightly increased water swelling index and water binding index, (c) reduced the bulk density, and (d) the stabilizer Tween 80 improved the overrun of the whipped product.

Fish protein concentrate prepared by solvent extraction is a high-quality animal protein (80-95% protein), suitable to incorporate into many types of food and food products. With most protein products, it is assumed that quality is associated with functional properties. However, as fish protein concentrate has poor functional properties, its use by food industries is limited. Improvement of its functional properties could greatly expand its usefulness. FPC is a relatively new protein and there is little information available on the functional properties of this product.

Much work has been done to explain the functional properties of other proteins such as egg albumin. Zabik (15) used oil in water systems to study the emulsifying properties of frozen and spray-dried whole eggs. Brown and Zabik (3) investigated the separate and additive effects of preheating per se, and of preheating in conjunction with spray drying on the functional properties of egg albumin. They found a decrease in foam stability and apparent surface tension although specific gravity was increased. On the other hand, Ayers and Solosberg (2) reported that pasteurization did not affect the functional properties of dried egg albumin.

Spray drying has been used with great success in the food industry. Over a billion pounds of milk are spray dried annually, and the annual production of other spray-dried foods, such as coffee, runs into the hundreds of millions of pounds (11). Acceptance of

spray drying is attributed to the favorable characteristics of food products and to the economics of operation. Franks et al. (6) investigated the performance and quality characteristics of spray-dried and freeze-dried egg albumin in angel cake. They found all cakes prepared from these products received good to very good scores for quality characteristics.

The commercial production of freeze-dried food products has become a fact only during the past decade. Van Arsdell (14), Connel (5), and Hamdy et al. (8) pointed out that the water-holding capacity of muscle protein was lost by freeze drying. In animal protein the loss of water-holding capacity is assumed to be due to increased cross-linkage of the myofibrillar proteins, primarily actomyosin (10).

Thus the purposes of this study were (a) to explore and measure the functional properties of FPC, (b) to study the effect of further processing such as spray drying and freeze drying on functional properties of FPC, and (c) to study the effect of some commercial stabilizers on the functional properties of FPC, as prepared by isopropyl alcohol extraction and after further processing.

EXPERIMENTAL

Materials

FPC was prepared from whole hake (*Urophycis chuss*) by solvent extraction with isopropyl alcohol (4). The FPC was ground by a Rietz disintegrator and steam stripped at 2-3 lb. pressure for 10 min.¹ The FPC was analyzed for chemical composition (1) and its analysis was as follows: protein, 83%; volatiles, 6%; ash, 14%; lipids, 0.3% and residual isopropyl alcohol, <90 ppm. The sample was analyzed for particle size using a Taylor testing sieve unit. The particle size distribution of the FPC is shown in Table 1. Soy flour and soy isolate (Promine D) were obtained from Central Soya (Chemurgy Division, Chicago).

TABLE 1. PARTICLE SIZE ANALYSIS OF HAKE-FPC

Particle size (Microns)	% by weight
<125	100
<125 >88	22
< 88 >53	39.5
<53	38.5

¹Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 2. EFFECT OF FREEZE DRYING AND SPRAY DRYING WITH AND WITHOUT STABILIZERS ON CERTAIN FUNCTIONAL PROPERTIES OF FPC MADE FROM HAKE COMPARED TO SOY FLOUR AND SOY ISOLATE

Protein	pH	Bulk density (wt/v)	Wettability (sec)	Suspended solids (%)	Soluble protein (%)	Emulsion capacity (ml oil/g protein)
<i>Freeze-dried FPC</i>						
FPC						
Stabilizers added						
Myvoplex 600	7.4	0.35	4.7	6.3	7.1	2.7
Tween 80	7.5	0.33	1.7	7.4	3.5	9.3
CMC	7.5	0.37	2.2	9.1	3.9	9.6
Alcobic 505	7.5	0.38	6.0	7.8	5.7	8.9
No stabilizer	7.6	0.37	2.2	5.0	3.4	8.0
<i>Spray-dried FPC</i>						
FPC						
Stabilizers added						
Myvoplex 600	7.7	0.44	14.0	4.8	5.7	2.1
Tween 80	7.6	0.43	2.7	5.5	3.3	8.4
CMC	7.6	0.46	4.2	6.6	3.7	5.8
Alcobic 505	7.5	0.49	6.7	5.8	4.4	8.6
No stabilizer	7.7	0.44	3.1	4.5	2.3	2.7
<i>Untreated</i>						
FPC (control)	7.6	0.52	— ¹	6.1	2.6	5.8
Soy flour	6.8	0.52	68	57.1	9.6	20.0
Soy isolate	7.3	0.38	743	43.5	14.6	48.8

¹Not wettable.

Treatments

A slurry was made using 150 g of FPC and 650 ml of distilled water at room temperature using a Waring blender. On the basis of the dry FPC, 2% of four commercial stabilizers was added to separate samples and the samples were blended for 1 min.² One set of treated samples was then freeze dried at 104 F, and another set was spray dried with an outlet temperature of 160 ± 5 F and 160 lb./in² pressure.

Functionality measurement

Functionality measurements were made on both sets of samples, on untreated FPC as the control, soy flour, and soy isolate. The samples were evaluated for pH, bulk density, wettability, suspended solids, soluble protein, water swelling index, water binding index, overrun, emulsion capacity, and emulsion stability. These parameters also were evaluated on soy flour and Promine D (soy isolate) and compared with the experimental FPC samples and the control.

pH. The pH was measured by making a 5% dispersion of each sample in distilled water and by blending for 30 sec in a Waring blender.

Bulk density. The moisture content was equilibrated before the bulk density was measured (12). A 25-ml graduated cylinder with an opening of 1 cm² was gently filled by means of a funnel. Bulk density was calculated by dividing the volume (25 ml) into the weight of the sample. The measurement of bulk density for each sample is the average of three analyses and standard deviation was about 0.01.

Wettability. One gram of sample was placed in a 25-ml graduated cylinder with an opening of 1 cm². A finger was

placed over the open end and the cylinder was inverted and clamped at a height of 10 cm above the surface of a 600-ml beaker containing tap water. The finger was released, and the time required for the sample to get wet completely was measured with a stop watch.

Suspended solids. A 2% dispersion of the sample in distilled water was made in a 100-ml graduated cylinder. The cylinder was capped with a rubber stopper and then it was shaken in a horizontal Dubnoff shaker (Precision Scientific) for 0.5 h. The cylinder was removed from the shaker and allowed to stand for 2 h. A 5-ml aliquot of the supernate was removed from the midpoint of the cylinder by a pipette, transferred to an aluminum dish, and dried in an oven overnight at 100 C. The total suspended solids was calculated as a percentage of the total weight of FPC sample.

Salt soluble protein. A 2-g sample was blended with 100 ml of 5% NaCl solution for 2 min, then it was centrifuged at 3000 g for 30 min. The supernate was evaluated for protein (N × 6.25) using AOAC method 2.051 (1).

Emulsion capacity. A 1-g sample was mixed with 34 ml of 3% NaCl solution in a Waring blender for 30 sec. While continuing blending, 30 ml of Wesson oil were added at the rate of 10 ml/min and blending continued for an additional 30 sec. The sample was transferred to a 50-ml graduated centrifuge tube. The centrifuge tube was kept in a water bath at 80 C for 15 min and then centrifuged at 3000 g for 30 min. The volume of oil separated from the sample after centrifugation was measured in a graduated cylinder. Emulsion capacity was expressed as the amount of oil emulsified and held per gram of FPC.

Emulsion stability. A 1-g sample was dispersed in 25 ml of distilled water and then 25 ml of Wesson oil were added at a rate of 25 ml/min while blending. The sample was blended at high speed in a Waring blender for an additional 2 min and transferred into a graduated cylinder. Volumetric changes in the foam, oil phase, and water phase were recorded after 0.5 and 2 h.

²The stabilizers used in this experiment were as follows: (a) Tween 80 (polysorbate), Atlas Chemical Co., Wilmington, Delaware; (b) Alcobic 505 (lecithin), American Lecithin Company, New York, N. Y.; (c) Myvoplex 600 (glycerol monostearate), Distillation Products, Rochester, N. Y.; and (d) CMC (cellulose gum), Hercules, Inc., Wilmington, Delaware.

TABLE 3. EFFECT OF FREEZE DRYING OR SPRAY DRYING WITH AND WITHOUT STABILIZERS ON THE SWELLING IN WATER INDEX AND WATER BINDING INDEX OF FPC MADE FROM HAKE COMPARED TO SOY FLOUR AND SOY ISOLATE

Protein	(A)	Suspended solids	(B)	(C)	Water swelling index	Water binding index ¹
	Swelled volume		Water bound	Insoluble fraction		
	(ml)	(g)	(g)	(g)	A/C	B/C
FPC						
					<i>Freeze-dried FPC</i>	
Stabilizers added						
Myvoplex 600	4.9	0.12	3.3	0.89	5.6	3.7
Tween 80	4.7	0.14	3.1	0.86	5.5	3.6
CMC	4.3	0.13	3.0	0.87	5.3	3.4
Alcobic 505	4.6	0.12	3.0	0.88	5.3	3.4
No stabilizer	5.1	0.10	3.4	0.89	5.7	3.8
					<i>Spray-dried FPC</i>	
FPC						
Stabilizers added						
Myvoplex 600	4.2	0.10	3.3	0.90	4.7	3.6
Tween 80	4.2	0.12	3.1	0.88	4.8	3.5
CMC	4.0	0.11	3.1	0.88	4.5	3.5
Alcobic 505	4.0	0.11	3.1	0.90	4.4	3.4
No stabilizer	4.3	0.10	3.2	0.90	4.8	3.6
					<i>Untreated</i>	
FPC (control)	3.7	0.12	3.0	0.88	4.3	3.4
Soy flour	4.9	0.66	2.9	0.33	14.8	8.7
Soy isolate	6.3	0.88	4.9	0.16	38.2	30.6

Water swelling index and water binding index. A 1-g sample was dispersed in distilled water to a total volume of 10 ml in a graduated centrifuge tube, mixed with a glass rod and shaken vigorously for at least 3 min to assure complete wetting. The sample was allowed to settle at room temperature for 2 h and the volume of the precipitate recorded (swelled volume). The supernate was separated from the precipitated solids by the aid of a Hamilton syringe, gently transferred to an aluminum dish, dried overnight in the oven at a temperature of 100 C and weighed (suspended solids). The weight of insoluble solids was calculated by subtracting the weight of suspended solids from the weight of original sample. The weight of the water bound was measured after drying the insoluble fraction in an oven at 90 C overnight. Functional parameters were calculated by the following formula:

$$\text{Water swelling index} = \frac{\text{swelled volume}}{\text{weight of insoluble fraction}}$$

$$\text{Water binding index} = \frac{\text{weight of water bound}}{\text{weight of insoluble fraction}}$$

Overrun. A 1-g sample was blended with 90 ml of distilled water at high speed (1500 rpm) in a Waring blender for 2 min. After blending the sample was transferred in a 150-ml graduated cylinder. The blender jar was washed with 10 ml of distilled water and it was added to the graduated cylinder. For each treatment three samples were prepared and they were blended for 4, 6, and 8 min and the increase in the volume after blending was recorded. The percent overrun was calculated as follows:

$$\% \text{ overrun} = \frac{(\text{Volume after whipping} - \text{volume before whipping})}{\text{volume before whipping}} \times 100$$

To a second set of samples, 25 g of commercial sucrose were added to each sample and the percent overrun was measured at 2-min intervals. To a third set of samples, 10 drops of Wesson oil were added to each sample and percent overrun was measured.

RESULTS

The results of the measurement of pH, bulk density, wettability, suspended solids, soluble protein, and emulsion capacity on the samples and controls are given in Table 2.

pH

There were minor differences in the pH values of the various samples. All FPC samples had pH values close to 7.0, similar to soy flour and soy isolate.

Bulk density

Freeze-dried samples all had lower bulk density than the spray-dried samples, and processed samples had lower bulk density than the control sample and soy flour but were similar to soy isolate. The various stabilizers had little effect on the bulk density.

Wettability

All freeze-dried and spray-dried samples were completely wettable, whereas the control sample stayed on the surface and did not wet in water. In general, freeze-dried samples required less time to get wet than did spray-dried samples. Freeze-dried and spray-dried FPC samples were more wettable than soy isolate or soy flour. The stabilizers did not improve the wettability of the samples with the exception of Tween 80, and that improvement was slight.

Suspended solids

The suspended solids for the freeze-dried samples were slightly higher than spray-dried samples. When stabilizers were added, slightly higher values were

TABLE 4. EFFECT OF FREEZE DRYING AND SPRAY DRYING WITH AND WITHOUT STABILIZERS AND WITH AND WITHOUT ADDED SUCROSE OR OIL ON THE PERCENT OVERRUN OF FPC MADE FROM HAKE COMPARED TO SOY FLOUR AND SOY ISOLATE

Protein	1% FPC			1% FPC + 25 g sucrose			1% FPC + 10 drops Wesson oil		
	time (min)			time (min)			time (min)		
	4	6	8	4	6	8	4	6	8
	(% overrun)								
<i>Freeze-dried FPC</i>									
FPC									
Stabilizers added									
Myvoplex 600	1.0	1.0	1.0	4.5	4.5	1.0	2.0	0.0	0.0
Tween 80	52.5	52.5	50.5	60.7	50.0	55.3	9.8	7.8	7.8
CMC	10.9	10.9	10.9	15.0	9.0	16.0	2.0	2.0	2.0
Alcobic 505	12.9	8.9	10.9	3.6	1.0	7.1	0.9	0.0	0.0
No stabilizer	28.7	33.7	28.7	20.5	16.1	16.1	2.0	2.0	2.0
<i>Spray-dried FPC</i>									
FPC									
Stabilizers added									
Myvoplex 600	2.0	5.0	3.0	5.3	3.6	3.6	2.0	3.0	1.0
Tween 80	58.4	62.4	60.4	58.9	62.5	66.0	7.8	5.9	5.9
CMC	12.9	12.9	13.9	14.3	17.9	16.1	1.0	1.0	1.0
Alcobic 505	11.0	10.9	8.9	7.2	5.3	8.9	2.0	1.0	2.0
No stabilizer	22.8	18.8	20.8	19.6	7.2	7.2	2.0	1.0	1.0
<i>Untreated</i>									
FPC (control)	21.6	23.5	25.5	37.3	13.7	13.7	2.0	4.0	4.0
Soy flour	8.9	23.7	18.7	7.2	7.2	15.1	18.2	28.2	23.2
Soy isolate	23.7	28.7	28.7	15.1	23.0	31.0	23.2	28.1	33.0

TABLE 5. EMULSION STABILITY OF FREEZE-DRIED AND SPRAY-DRIED HAKE FPC AFTER TREATMENT WITH STABILIZERS COMPARED WITH SOY FLOUR AND SOY ISOLATE

Protein	Volume (ml) after 1/2 hours				Volume (ml) after 2 hours				
	Foam	Oil	Aqueous	Total	Foam	Oil	Aqueous	Total	
<i>Freeze-dried FPC</i>									
FPC									
Stabilizers added									
Myvoplex 600	0	34	64	98	0	31	66	99	
Tween 80	6	32	64	102	6	25	65	90	
CMC	2	34	63	99	0	32	65	97	
Alcobic 505	3	30	68	100	3	28	70	98	
No stabilizer	4	28	68	100	2	25	70	95	
<i>Spray-dried FPC</i>									
FPC									
Stabilizers added									
Myvoplex 600	2	28	62	92	2	28	61	91	
Tween 80	5	27	62	94	3	27	64	94	
CMC	3	28	60	91	2	28	64	94	
Alcobic 505	5	26	63	94	4	26	63	93	
No stabilizer	3	30	60	93	3	30	60	93	
<i>Untreated</i>									
FPC (control)	2	36	63	101	0	32	68	100	
Soy flour	0	0	0	101	5	40	59	101	
Soy isolate	10	0	0	110	8	27	68	103	

obtained with freeze-dried samples than with the control sample. All samples had substantially lower suspended solid values than the soy flour and soy isolate.

Soluble protein

The soluble protein ranged from 2-3% for the sample that was spray dried only to 7.1 for the freeze-dried sample with Myvoplex 600. All FPC samples gave values lower than soy flour and soy isolate.

Emulsion capacity

Emulsion capacity was improved slightly by freeze drying alone but not by spray drying alone. However, those samples that were treated by Tween 80 and Alcobic 505 had higher emulsion capacity. CMC improved emulsion capacity of the freeze-dried sample but not the spray-dried sample. All FPC samples had lower emulsion capacity than soy flour and soy isolate.

Emulsion stability

Table 5 shows that all FPC samples had very poor emulsion stability. The emulsions separated into different phases after 0.5 h, and only slight changes were observed between 0.5 and 2 h. The total volume of the spray-dried samples was slightly lower than that of the freeze-dried samples.

Water swelling index and water binding index

Table 3 shows that the water swelling index and water binding index were both improved by either freeze drying or spray drying. Spray-dried samples gave higher values for the water swelling index than freeze-dried samples, but the values for water binding index were practically the same. The addition of stabilizers did not increase either index; in fact, slight reductions were noted with several of the stabilizers.

Overrun

Table 4 shows that freeze drying or spray drying did not substantially improve the overrun of the sample, however, it was affected by certain stabilizers. The values for overrun for the samples with Myvoplex 600, Alcobic 505, and CMC were lower than the control sample when no sucrose was used, but with Tween 80 the overrun was increased two- to three-fold. In general, similar results were obtained when 26 g of sucrose were added to each sample. For the control sample the percent of overrun was increased at the beginning of blending (after 4 min) then it was decreased. Samples with Tween 80 and sucrose had the highest increase in overrun, more than twice the value for soy flour or soy isolate. The overrun for all FPC samples was decreased when Wesson oil was added to the samples.

Freeze drying and spray drying change the hydrophobic character of FPC enabling it to wet more readily, presumably because rapid freezing forms ice crystals that leave pores between particles, changing the physical properties of the product so that it will reconstitute easily (7). Spray-dried products, on the other hand, have a microporous nature capable of providing rapid diffusion of water into the interior of the particles. As a result, both freeze-dried and spray-dried FPC have lower bulk density and a higher water-binding capacity confirmed by the higher water binding index.

Joslin and Proctor (9), Rolfes et al. (13) and Zabik and Figa (16) previously reported that freeze-dried whole egg has poor foam-forming ability. This was also true for all spray-dried and freeze-dried FPC samples except those treated with Tween 80 (Table

5). The overall conclusions from this study were that: (a) both spray drying and freeze drying resulted in a total wettable FPC product; however, neither spray drying nor freeze drying improves overrun or emulsion stability; (b) spray drying and freeze drying both resulted in lowering the bulk density of FPC, freeze drying being particularly effective; and (c) among the stabilizers, Tween 80 was the only one effective in increasing the overrun after whipping.

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AN OCCUPATIONAL SAFETY AND HEALTH STATE PLAN¹

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ABSTRACT

The reasons for the Occupational Safety and Health Act are reviewed, including a brief summary of the Congressional action incident to passage of the Act. The declared purpose of Congress is delineated in the 13 ways proposed to achieve its purpose to assure, so far as possible, safe and healthful working conditions for every employee in America and to preserve this country's human resources. Coverage of the Act and some aspects of its implementation are considered. The basic consideration of developing and implementing a State Plan are outlined including some of the disconcerting problems involved.

Not unlike many of our public health laws that are responsive to the needs of all people, safety and health laws, historically, have been left to the states and their legislative actions. Hence the laws were a response relative to problems of the particular state and their application was befitting the circumstances in that state.

A few industrialized states did develop safety and health programs and supporting laws in an effort to reduce deaths and control accidents and injuries in the workplace. There was, however, a great deal of frustration within those states that were bordered by states who saw no need for comparable programs or legislation.

Finally, in 1970, such statistics as almost 15,000 deaths and hundreds of thousands of serious injuries in the workplaces of the United States billowed up in Congress like a smoke signal. Action began to develop. As is true of most important legislation, there quickly developed differences of opinion in interest and philosophy which involved both party politics and labor-management considerations. Among the notables in the House of Representatives were Congressmen Hathaway, O'Hara, Perkins, Steiger, Sikes, and Daniels. In the Senate there were Williams, Javits, and Dominick.

Finally, the joint committee met in five sessions to work out the difference between the bills from the House and Senate. After brief debate the conference report was approved by both legislative bodies. On December 29, 1970, President Nixon signed into law the Occupational Safety and Health Act of 1970.

The ultimate success or failure of the Act is dependent on the understanding, the belief in, and the implementation of the Act so the objectives expressed by the Congress will be achieved. Congress felt that work-related personal injuries and illnesses imposed a substantial burden on and hindered interstate commerce by causing losses in production and wages and through medical expenses and disability payments.

THE INTENT OF CONGRESS

Congress declared it to be its purpose and the policy of the nation to assure *so far as possible safe and healthful working conditions to every employee in America and to preserve this country's human resources*. For the record I am quoting the intent of the Congress to achieve its purpose in the following ways:

- (a) By encouraging employers and employees in their efforts to reduce the number of occupational safety and health hazards at their places of employment and to stimulate employers and employees to institute new and to perfect existing programs for providing safe and healthful working conditions;
- (b) By providing that employers and employees have separate but dependent responsibilities and rights with respect to achieving safe and healthful working conditions;
- (c) By authorizing the Secretary of Labor to set mandatory Occupational Safety and Health Standards applicable to businesses affecting interstate commerce, and by creating an Occupational Safety and Health Review Commission for carrying out adjudicatory functions under the Act;
- (d) By building upon advances already made through employer and employee initiative for providing safe and healthful working conditions;
- (e) By providing for research in the field of occupational safety and health including the psychological factors involved, and by developing innovative methods, techniques, and approaches for dealing with occupational safety and health problems;
- (f) By exploring ways to discover latent diseases, establishing casual connections between diseases and work in environmental condition, and conducting other research relating to health problems, in recognition of the fact that occupational health standards present problems often different from those involved in occupational safety;
- (g) By providing medical criteria which will assure insofar as practicable that no employee will suffer diminished health, functional capacity, or life expectancy as a result of his work experience;
- (h) By providing for training programs to increase the

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number and competence of personnel engaged in the field of occupational safety and health;

- (i) By providing for the development and promulgation of occupational safety and health standards;
- (j) By providing an effective enforcement program which shall include a prohibition against giving advance notice of any inspection and sanctions for any individual violating this prohibition;
- (k) By encouraging the States to assume the fullest responsibility for the administration and enforcement of their occupational safety and health laws by providing grants to the States to assist in identifying their needs and responsibilities in the area of occupational safety and health, to develop plans in accordance with provisions of this Act, to improve the administration and enforcement of State occupational safety and health laws, and to conduct experimental and demonstration projects in connection therewith;
- (l) By providing for appropriate reporting procedures with respect to occupational safety and health which procedures will help achieve the objectives of this Act and accurately describe the nature of the occupational safety and health problems.
- (m) By encouraging joint labor-management efforts to reduce injuries and disease arising out of employment."

There is no question that if these objectives can be carried out—wonders in the workplace will be wrought and many lives will be saved and much lost time injury will be avoided. Objectives k, l, and m are the key areas in which the States have undertaken development and proposed implementation of the Williams-Steiger Occupational Safety and Health Act of 1970.

DEVELOPING A STATE PLAN

Coverage of the Act is quite broad and is still under fire in many quarters. But in general, the definition of employer and employee from a legal point of view is inclusive with few exceptions. These few exceptions include: domestic help in a private home and the immediate family of a farm family operation, also public employees are excluded from coverage of the Act. In developing a State Plan acceptable to the U. S. Labor Department, under the terms of the Act, a State is obliged to consider many ramifications and applications, among which is, as a general duty in structuring its program and legislation, to realize that the common law concept—that a person must refrain from actions which cause harm to others — is part and parcel of the Federal Act and must be carried into the action and legislation of any State program.

Other aspects to be considered are (a) the Standards. Standards would, of necessity, be required as effective as those of the Federal Standards. (b) Enforcement. Enforcement likewise needs to be as effective as though it is carried on by Federal compliance personnel and administrative procedure. (c) There must be a system of administrative review of

the various citations when undertaken at the State level. (d) Also, some form of legal or judicial review is necessary as part of the State plan and support legislation. (e) To know where we have been and where we are going, records, statistics and reports will be a part of the basic program and need to be designed so that they can be responsive to the needs of the particular state. (f) A great deal of research is necessary since, very quickly, it was realized that the U. S. Labor Department under the Assistant Secretary for Occupational Safety and Health was learning as it attempted to implement Public Law 91-596. There has been great diligence in the attempt of the U. S. Labor Department to work out its plans to assist the various States in their efforts to assume the responsibility which could be delegated to them by authority of the Act but this monumental responsibility and effort has been interrupted by a change of Secretaries and circumstances within the department which have materially slowed down the process of approval and implementation of State Plans.

STEPS IN DEVELOPING THE PLAN

Speaking for the States where only a limited activity in occupational safety and health was in effect, it was almost like starting from scratch.

The steps in the development of the Plan are essentially these: *First*, the Governor of the State appoints a designee, a department or agency within the State that becomes the responsible occupational safety and health agency to ultimately enforce and implement the Act at the State level.

Second, the State agency, in most instances under a grant, as authorized under the Act, will do research and evaluation of their State needs.

Third, following this exploration, in most instances, additional monies are provided on a matching basis from the Federal government for developing plans and procedures to actually assume responsibility at the State level. These plans will, of necessity, require the following basic considerations:

- (a) An adequate administrative staff which will include the necessary administrative assistants and clerical back-up along with the expertise needed for a standards and training capability for the ongoing program to insure updating and review of standards as well as compliance procedures.
- (b) A strong and effective compliance department is required by the Act to enforce the regulations promulgated and adopted under legal authority within the State.
- (c) In connection with the enforcement process, to be acceptable, it is paramount that there be established a Review Board and an advisory committee. In most instances these people will be appointed by the Governor of the State and approved by its legislature, either in the House or Senate or the appropriate body within its structure.

- (d) Under the Act there will be, of necessity, some effort to establish comparable rules and regulations "as permitted by State law" to protect public employees within that individual state.
- (e) The next facet of any State program will be that of statistical competence. This effort is developed through the Bureau of Labor Statistics. Hopefully in development of statistical information and management information systems (MIS), we, at the State level, will be able to ascertain our needs and develop priorities for activity that will reduce the number and severity of injuries as well as number of fatalities.
- (f) The other area open to the States within their planned implementation of the Federal Act is authorization by the U. S. Labor Department to develop a technical service and training section for the State operation. In our Plan, we refer to it as our Department of Technical Services and Education. This activity must be kept separate from the compliance group (enforcement group), as referred to in the Federal activity. This is based on the fact that one of the means by which Congress expected these laws to be effective reads (Item 10, in part),—"shall include a prohibition against giving advance notice of any inspection and sanctions for any individual violating this prohibiton."

In Mississippi all responsibility for occupational safety and health is vested in the Division of Occupational Safety and Health. In most other states there is a Department of Labor or similar department which is the designee. Matters of training and education, the industrial hygiene, and the health facility are contracted to another department of the State as is statistical activity. This permits the designee to be less concerned with overlap of certain functions and also gives the designee the peculiar advantageous situation in that all policy and procedure can be directed more expeditiously and objectively without the problem of coordinating interagency prerogatives and administrative problems.

THE REVIEW PROCESS

One of the major problems in developing an adequate Plan and functional procedure has been the review process through which the States must develop such a plan. I presume, of necessity, the Labor Department has had to fragment its expertise in the review of plans. Some of those reviewers (exercising considerable authority) often seem in-

experienced though articulate individuals who do not appear to have an overview of the total plan as it will be applied within the State, nor do they appear to have an appreciation of the problems or peculiar circumstances within the State that will influence administration of the Plan under its support legislation. It has been somewhat like dealing with a biological research team, each individual looking through an individual microscope in which each expert sees only a small field for which they are responsible and really not relating the individual part to the whole problem which would be the State's in its actual enforcement and implementation of the Plan. As I indicated, this in all probability, is due to the required expertise of the individual in each area of concern but, objectively, it has made it difficult for the States to communicate with the Labor Department at the Federal level in an expeditious manner toward getting on with approval of the Plans and, in fact, implementing the necessary action at the State level which would materially speed up the capacity to reduce accidents and injuries as was the intention of the Act when passed.

Plan approval at the regional level would, where full oversight exist and understanding of the capacity of the State Designee to perform, serve to expedite the intent and purpose of OSHA (PL 91-596).

The fact that there has been this delay at the Federal level has created considerable anxiety and doubt in the minds of our adversaries (opposing State Authority) as to the real intention of the U. S. Labor Department to transfer responsibility as is the expressed intention of the Act. This situation has made it difficult for the designated agencies in certain States to sell their legislature on the appropriate support legislation necessary to implement the Act and to transfer the full responsibility to the State designee.

There is no question that the Occupational Safety and Health Act is desirable and should be implemented with full speed. There are serious conditions in every State that need attention and any procrastination and delay either at Federal or State level is a serious disservice to the employees and employers of this nation.

STATUS OF THE RADAPPERTIZATION OF MEATS¹

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ABSTRACT

Considerable progress has been made toward development of highly acceptable radappertized meats through application of a heat treatment to an internal temperature of 65-80 C to inactivate proteolytic enzymes before irradiation, low temperature (-30 ± 10 C) of the food during irradiation, and addition of low levels of tripolyphosphate and sodium chloride. To assure that radappertized meats are free of potential pathogens and spoilage microorganisms they are given a minimum radiation dose (MRD) computed to effect a 12 log cycle reduction in the most radiation resistant strains of *Clostridium botulinum* spores. Inoculated pack studies are carried out to obtain the specific microbiological data required for computing the MRD. Cured meats normally have a lower MRD than uncured meats. In model systems concentrations of sodium chloride (NaCl) up to 4.0% (w/v) present during irradiation had no effect on radiation resistance, but NaCl did inhibit recovery of irradiated spores. A mixture of salts (4.0% NaCl, 30 ppm NaNO₂, 500 ppm NaNO₃) had essentially the same effect as NaCl alone. Of 11 genera of vegetative cells examined, *Micrococcus radiodurans* and *Streptococcus faecium* were shown to be the most resistant to low-temperature gamma irradiation. Before the radappertization process can be established commercially it is necessary to provide proof that products so treated are safe for human consumption. An intensive animal feeding study of radappertized (4.7-7.1 Mrads) beef was initiated in 1971 and is expected to be completed in 1976.

Since 1954, the U. S. Army and the Atomic Energy Commission (AEC) have conducted or sponsored most of the research and development on food irradiation within the United States. The AEC has been attempting to increase the refrigerated shelf life of various fruits, vegetables, fin fish, shellfish, and poultry by applying submegalrad doses. The AEC plans to submit petitions to the U. S. Food and Drug Administration (FDA) for approval of irradiated strawberries and papayas. Strawberries are irradiated with low doses to reduce the mold population and thus extend shelf life whereas papayas are exposed to irradiation to destroy insect infestation and thereby remove present quarantine barriers. On the other hand, the U. S. Army's food irradiation program has as its primary objective radappertization (19) of packaged meats. In this process megalrad doses are used to prevent any spoilage or toxicity of microbial

origin during several years of storage without refrigeration.

TECHNOLOGY AND ACCEPTANCE

Before irradiation, raw meat is heated to an internal temperature of 65 to 80 C to inactivate radiation-resistant proteolytic enzymes. The enzyme-inactivated meat is vacuum packed in metal cans or in flexible pouches, frozen, and irradiated at -30 ± 10 C with gamma rays from a cobalt-60 source or with electrons using a linear accelerator. Irradiation at -30 ± 10 C results in a marked improvement in quality over ambient temperature irradiation for most products (43). The improvements are significant in nearly all factors including reduction in off-color, off-odor and irradiation flavor, and minimizing loss of texture. Improvements in juiciness of irradiated beef, pork, and chicken have been realized by adding to the meats, before enzyme inactivation, 0.5% to 1.0% sodium chloride and 0.3 to 0.5% sodium tripolyphosphate (40). These salts reduce weight loss (loss of natural juices) during cooking from 30-35% to 10-15%.

Preference scores based on the 9-point hedonic scale (32), show that most radappertized meats, poultry, and seafoods are highly acceptable. Irradiated chicken parts (23) and codfish cake (24) were shown to compare favorably with the frozen unirradiated control. During the flight of Apollo 17 (December 1972) each of the three astronauts consumed three ham sandwiches prepared in cooperation with the University of Nebraska from ham radappertized (3.7-4.3 Mrad) at the U. S. Army Natick Laboratories (NLABS) and rye bread radurized (19) at the University of Nebraska with 40-50 krad to reduce or eliminate dominant spoilage microbes. Each of the astronauts gave the ham sandwiches an excellent rating (11). The ham was packaged in flexible pouches. No moisture or meat juice droplets were permitted in the ham pouches because, in the weightless conditions of space, droplets could be inhaled by the astronauts or could float into electronic switches and components and jeopardize the mission. Not only was the moisture content of the ham carefully controlled, but, in addition, a surgical gauze pad was

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TABLE 1. INOCULATED PACK PROCEDURE FOR RADAPPERTIZATION OF FOODS

Prototype food	40-45g/container
Container	211 × 101.5 (epoxy enamel) can
Containers/dose	100 replicates
<i>C. botulinum</i> strains	33A, 36A, 62A, 77A, 12885A, 9B, 40B, 41B, 53B, 67B
Spore inoculum	10 ⁶ /strain, 10 ⁷ /can
Vacuum seal	125 mm mercury pressure
Radiation temperature	-30 ± 10 C
Doses (Mrad)	1.0-5.0 Mrad in increments of 0.5 Mrad or less
Incubation	6 Months at 30 ± 2 C
Analyses	Swelling, toxin, recoverable <i>C. botulinum</i>

sealed into the flexible pouch to absorb the unavoidable small amount of juice ("drip") that was produced during storage. Twenty-eight days of aerobic and anaerobic incubation at 32 ± 2 C and 55 ± 2 C followed by microbiological tests (22) showed the ham to be sterile.

INOCULATED PACKS

To assure the microbiological safety of irradiated foods, inoculated pack studies are done with each food. *Clostridium botulinum* spores, which were demonstrated to be the most resistant of the common foodborne pathogenic and spoilage microorganisms (18, 29, 30, 31, 34, 42), are used as the microbial index for determining the minimal radiation dose (MRD) required to assure the microbiological safety and stability of irradiated foods. The minimum radiation dose is presently defined as the dose that results in a reduction of the most radiation resistant strain of *C. botulinum* spores by 12 log cycles.

Inoculated pack studies are designed to provide partial spoilage data for computation of the MRD (12D dose). In the past both a screening and a clearance inoculated pack study were carried out to obtain the specific microbiological data for computing the MRD of each prototype food (36). The screening pack was designed to select from 10 strains of *C. botulinum*, five of high, four of intermediate and one of low radiation resistance (8), the one of greatest radiation resistance in a specific food product. Using the most resistant strain(s) a clearance pack was undertaken to provide the data for computation of the MRD. A new inoculated pack procedure (Table 1) is now being used to determine the MRD for beef containing condensed phosphate (0.4%) and small amounts of sodium chloride (0.75%). This procedure with which the FDA has concurred, represents a considerable savings in both time and cost. Each can (100 replicates/dose) containing 40-45 g of food is inoculated with 10⁶ spores of each of 10 strains of *C. botulinum* produced by the biphasic system (5), to give a total inoculum of 10⁷. Five of the strains are of high radiation resistance and five of intermediate resistance when compared with 102 strains previously screened (8). The cans are vacuum sealed (125 mm pressure) and irradiated at -30 ± 10 C. Following irradiation all cans are incubated at 30 ± 2 C for 6 months. During this period, containers are examined periodically for swelling. At the end of 6 months incubation, the examination for toxin and survivors begins at the highest dose yielding 100% swollen containers and includes all swollen and non-swollen cans at higher doses. Assuming exponential spore death in the cans, radiation D values, or dose

TABLE 2. MINIMAL RADIATION DOSE (MRD) AND EXPERIMENTAL STERILIZING DOSE (ESD) OF MILITARY PROTOTYPE FOODS

Food	Irradiation temp. (°C)	Most resistant <i>C. botulinum</i> strain	ESD ^a (Mrad)	Schmidt-Nank ^b		Spearman Karber ^c	Ref
				Range	Ave		
Bacon	5 to 25	33A ^d	1.5 < ESD ≤ 2.0	1.9-2.7	2.3	2.3	7
Codfish Cake	-30 ± 10	53B	2.5 < ESD ≤ 3.0	3.1-3.2	3.2	3.2	6
Corned Beef	-30 ± 10	77A	2.0 < ESD ≤ 2.5	2.4-2.6	2.5	2.4	6
Ham	5 to 25	12885A	3.0 < ESD ≤ 3.5	1.8-4.2	2.9	3.4	3
Pork Sausage	-30 ± 10	41B	1.5 < ESD ≤ 2.0	2.4	2.4	2.7	6
Pork	5 to 25	62A	3.0 < ESD ≤ 3.5	3.9-4.8	4.3	4.3	2

^aLowest dose at which there is no swelling, toxicity or survival.

^bD × 12 where $D = \frac{\text{radiation dose (Mrad)}}{\log M - \log s}$; D is the dose which destroys 90% of the total inoculum; M is the total inoculum organisms per sample unit times number of units; and S is the number of spoiled sample units (39).

^cD × 12 where $D = \frac{LD_{50}}{\log A - \log 0.69}$ and the $LD_{50} = t_u + \frac{d}{2} - d \sum_{i=1}^u P_i$, where t_u is the highest sublethal dose,

d is the dose increment used, u is the number of dose levels giving partial spoilage, and P_i is the percent of negative sample units; A is the initial number of organisms per sample unit (9, 38).

^dIn screening pack for the first inoculated pack (bacon) only 6 strains (33A, 36A, 12885A, 9B, 41B and 53B) of *C. botulinum* were tested.

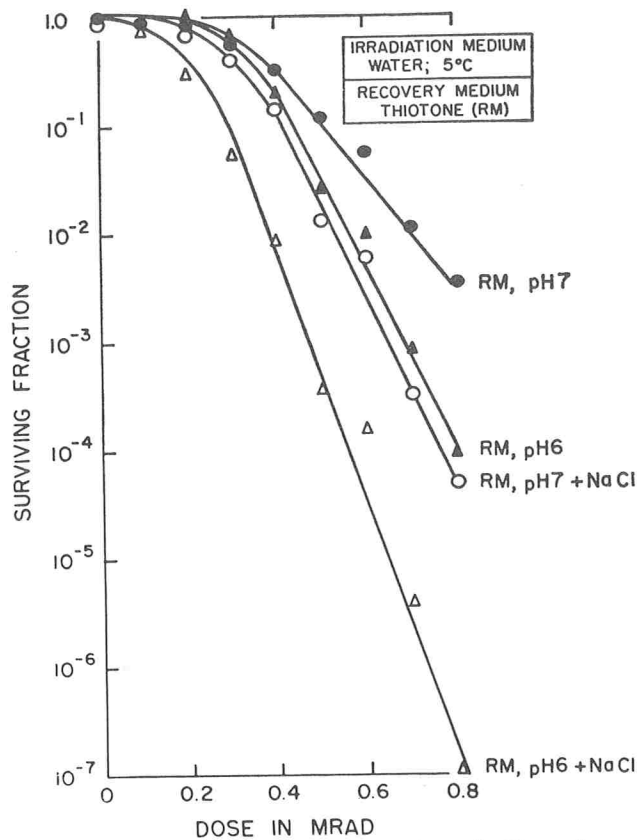


Figure 1. Effect of NaCl (40 mg/ml) and pH on the recovery (37 C) of irradiated (5 C \pm 2 C) *Clostridium botulinum* 62A spores (5×10^7 /ml glass distilled water). The recovery medium consisted of 3.0% BBL thiotone, 0.5% sodium thioglycollate, 0.85% oxoid ionagar no. 2, and 0.1% sodium bicarbonate.

for 90% destruction, are computed for the three criteria of spoilage: swelling, toxicity, and viable *C. botulinum* at each dose where partial spoilage is observed (3). The MRD is currently computed on the basis of spore survival regardless of their inability to outgrow or produce toxin in the meat. This MRD for survival is usually 0.5 to 1.5 Mrad higher than that computed for swelling or toxicity. Screening and clearance inoculated pack studies of 6 foods (2, 3, 6, 7) have been completed. Table 2 shows the MRD and experimental sterilizing dose (ESD) for each food. It is evident that the most resistant *C. botulinum* strain varies with the food under investigation. This emphasizes the importance of using several strains to determine the MRD of each food. The advantage and/or disadvantages of the Schmidt-Nank and Spearman-Karber methods of computing a MRD were previously discussed by Anellis and Werkowski (9).

INFLUENCE OF SODIUM CHLORIDE, SODIUM NITRATE, AND SODIUM NITRITE ON *C. BOTULINUM* IN THE RADIATION PROCESS

Those meats containing additives (e.g., sodium ni-

trate, sodium nitrite and/or sodium chloride) had notably lower MRD values than similar meats with no additives (Table 2). Whereas uncured pork (2) had an MRD of 4.3 Mrad, ham (3) containing, just before irradiation, 2.0 to 2.7 sodium chloride calculated as percent brine concentration, 222-309 ppm residual sodium nitrate and 1.7-6.0 ppm residual sodium nitrite, required 3.4 Mrad when irradiated at 5 to 25 C. Pork sausage (6) which contained 5-6% brine, and spices (black pepper, ginger, and sage) but no added nitrite and nitrate required only a dose of 2.7 Mrad at an irradiation temperature of -30 ± 10 C.

Studies in model systems were undertaken to determine what effect levels of curing salts detectable in meats just before irradiation had on the radiation resistance and recovery of *C. botulinum* 62A spores. In these experiments spores were suspended in water, NaCl, NaNO₃, NaNO₂ or combinations of the three salts, and exposed to gamma irradiation at 5 ± 2 C. After irradiation at various dose levels spores were diluted and colony counts were made in thiotone agar

TABLE 3. RESISTANCE TO GAMMA RAYS OF SOME FOOD SPOILAGE GRAM-POSITIVE AND GRAM-NEGATIVE NONSPOROGENIC BACTERIA IRRADIATED AT -80 C

Organism	Highest survival dose (Mrad)	Lowest lethal dose (Mrad)
<i>Micrococcus radiodurans</i>	2.4	2.7
<i>Streptococcus faecium</i> (3 strains)	1.5	1.8
<i>Streptococcus faecium</i> FEC	0.9	1.2
<i>Streptococcus faecalis</i>	0.9	1.2
<i>Streptococcus faecalis</i> 1539	0.6	0.9
<i>Streptococcus lactis</i> 1467	0.3	0.6
<i>Staphylococcus aureus</i> (6 strains)	—	<0.3
<i>Lactobacillus casei</i>	—	<0.3
<i>Lactobacillus arabinosus</i> 1475	—	<0.3
<i>Alcaligenes faecalis</i> QMB 1483	0.6	0.9
<i>Escherichia coli</i> (5 strains)	—	<0.3
<i>Escherichia coli</i> 3543-66	0.3	0.6
<i>Aerobacter aerogenes</i> (2 strains)	—	<0.3
<i>Aerobacter aerogenes</i> 3228-65	0.3	0.6
<i>Salmonella</i> (2 species)	0.3	0.6
<i>Salmonella</i> (4 species)	—	<0.3
<i>Serratia marcescens</i> (2 strains)	—	<0.3
<i>Proteus vulgaris</i> QMB 1563	—	<0.3
<i>Pseudomonas aeruginosa</i> QMB 1468	—	<0.3

TABLE 4. IRRADIATED BEEF PROTOCOL: DIET GROUPS

<i>Basal diets</i>
Semipurified diet—rodents
Commercial ration—dogs
<i>Test diets</i> ^a
Frozen beef
Thermally processed beef ($F_0 = 5.8$)
⁶⁰ Co irradiated beef (4.7 - 7.1 Mrad)
10 MeV Electron irradiated beef (4.7 - 7.1 Mrad)

^aThe test diets contain 35% beef and 65% basal diet (dry weight). All beef is enzyme inactivated (65-71 C).

TABLE 5. DIETS FOR RODENTS

Constituents	Sempurified (percent)	Mixed ^a (percent)
Protein	20.0 ^b	20.0 (from beef)
Fat	10.0 ^c	15.0 (from beef)
Corn oil	5.0	0.6 ^d
Salt mix ^e	4.0	4.0
CaCO ₃	—	0.8 ^f
Cellulose	4.0	4.0
L-cystine	0.2	0.2
Glucose	27.4	26.7
Cornstarch	27.4	26.7
Vitamin mix	2.0	2.0

^aProtein and fat are to be supplied by beef (35% dry weight) and may vary from the figures shown. Exact composition will be derived from analytical data.

^bAdded as vitamin-free casein equal to the protein level supplied by beef in the mixed diet.

^cAdded as hydrogenated fat.

^dUnderlined components in the mixed diet are the basal semipurified diet components and are to comprise 65% (dry weight) of the mixed diet.

^eBernhart-Tomarelli Salt Mix (12).

^fCalculated to restore the Ca/P ratio to 1.2 (Beef: 12 mg Ca, 180 mg P + 94 mg P from TPP (tripolyphosphate). Salts: 900 mg Ca, 700 mg P).

with or without curing salts. These studies indicated that residual levels of NaNO₃ of 500 ppm and NaNO₂ of 30 ppm did not play a significant role in lowering the radiation dose required for inactivation of *C. botulinum* spores (37). It has been suggested (15, 25, 26) that in the reaction of nitrite with meat components a substance(s) may be formed which inhibits outgrowth of germinated spores. Since this is an unknown substance its ability to increase the effectiveness of gamma irradiation in a model system was not checked. Although 4.0% NaCl lowered the germination rate of untreated *C. botulinum* 62A spores and caused a lag in outgrowth of germinated spores it did not permanently inhibit the spores from undergoing germination and development to the point of forming macrocolonies (36). It was also shown that although 4.0% NaCl in an irradiated suspension did not sensitize *C. botulinum* spores to gamma irradiation, when added to a recovery medium (pH 7.0) it reduced the number of recoverable radiation survivors. Since irradiation did not enhance the inhibitory effect of NaCl on the rate of germination it was suggested that radiation-injured spores were able to germinate but not able to grow out in the presences of NaCl during the test period. A further decrease in recovery of radiation survivors in the presence of 4.0% NaCl was exhibited when the pH of the recovery medium was reduced from 7.0 to 6.0 (Fig. 1). Apparently, radiation damaged spores are sensitive to both NaCl and pH. Others have demonstrated that NaCl does not affect the radiation resistance of spores of *Clostridium sporogenes*, *Clostridium oedematiens* (35), *Bacillus*

pumilus, *Bacillus stearothermophilus*, *Bacillus subtilis* and *B. subtilis* var. *niger* (13). Spores of *B. subtilis* and *B. subtilis* var. *niger* were the only ones that did not show a reduction in post irradiation recovery in the presence of NaCl. Examination of mixed salts (4.0% NaCl, 30 ppm NaNO₂, 500 ppm NaNO₃) showed that when present in the irradiation medium they had little effect on radiation resistance (Fig. 2). In the recovery medium (pH 6.0) the combined salts were no more effective than NaCl alone. The extent of recovery of irradiated spores in the presence of mixed salts was dependent on the level of NaCl in the recovery medium (Fig. 3). The number of radiation survivors was reduced with 2.0% NaCl (20 mg/ml), the lowest level tested, and was progressively lowered with increased levels of NaCl. It is also apparent that radiation damaged spores were more sensitive to NaCl than were unirradiated spores. Sodium chloride does appear to play a significant role in the reduction

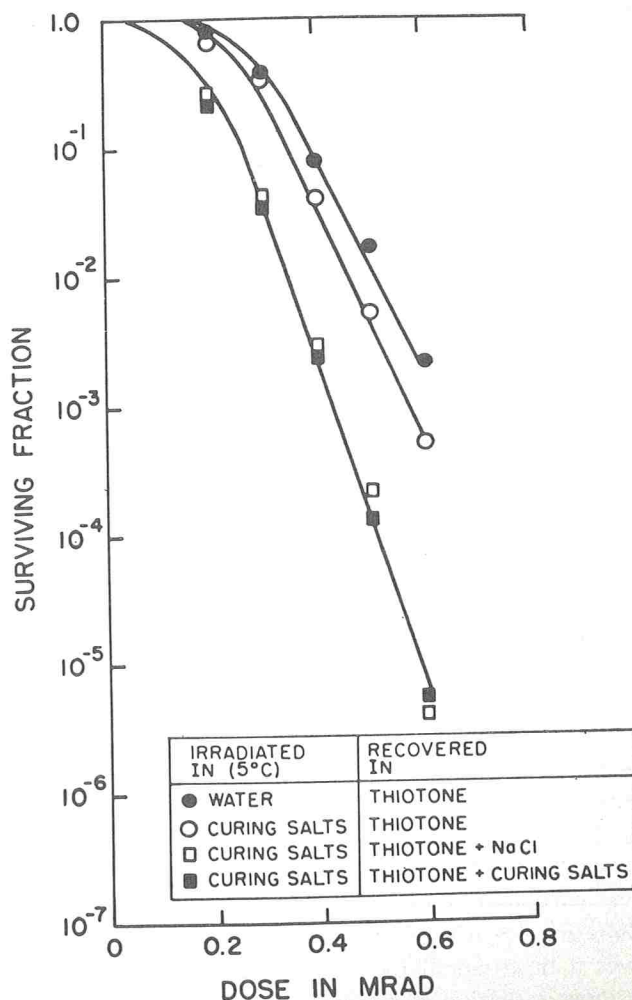


Figure 2. Effect of curing salts (NaCl, 40 mg/ml; NaNO₂, 30 µg/ml; NaNO₃, 500 µg/ml) on the radiation resistance and recovery (37°C, pH 6) of *Clostridium botulinum* 62A spores (5 × 10⁷/ml).

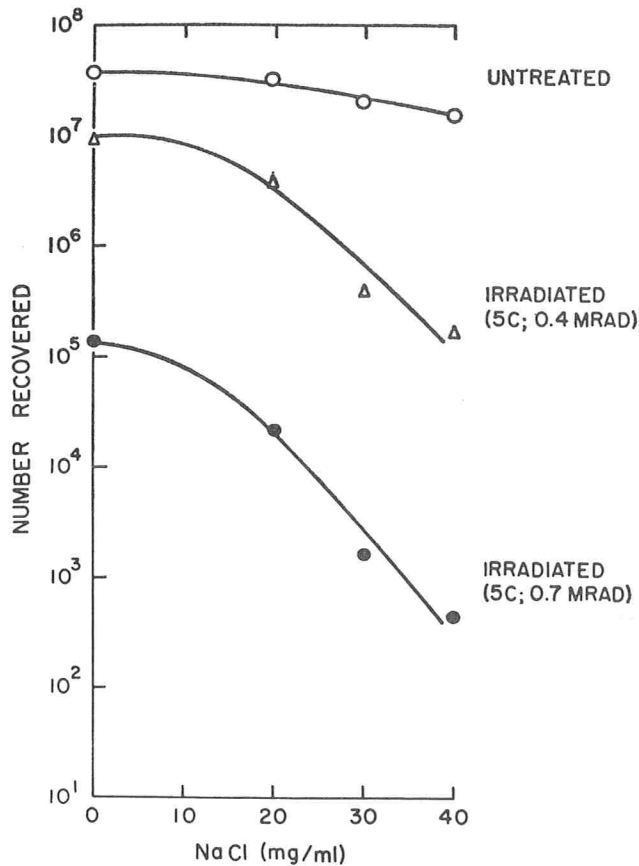


Figure 3. Effect of the level of NaCl in a thiotone medium on the recovery (37 C) of unirradiated and irradiated spores. Composition of the recovery medium (pH 6) was as indicated in Fig. 1 except for the addition of NaNO₂, 30 μg/ml; NaNO₃, 500 μg/ml; and levels of NaCl as shown.

of the radiation dose needed to effect a disappearance of surviving *C. botulinum* spores.

In the inoculated pack studies (e.g., ham) *C. botulinum* spores are exposed only to residual levels of NaNO₂ and NaNO₃ (3). Results of studies in model systems showed that these residual levels had little if any effect on lowering the radiation dose required for inactivation of *C. botulinum* spores (37). Thus, radappertized cured meats should not require high initial levels (156 ppm) of NaNO₂ to yield a product safe from hazards of *C. botulinum*. It was demonstrated that initial levels (150-200 ppm) rather than residual levels of nitrite play an important role in inhibiting toxin production by *C. botulinum* in perishable ham pasteurized to an internal temperature of 68.5 C (15). Perishable canned ham does not receive sufficient thermal processing to destroy *C. botulinum* spores whereas radappertized ham receives a dose calculated to destroy 12 log cycles of the most radiation resistant spores of *C. botulinum*. Although sodium nitrite may not be needed in radappertized ham to inhibit the growth of *C. botulinum* it is still required at low levels (25 ppm) for produc-

tion of the typical cured meat color, nitrosomyoglobin, and the typical cured meat flavor of ham (Wierbicki et al. 1973. Unpublished data). In addition, preliminary experiments indicate that sodium nitrate (50 to 100 ppm) in combination with the maximum allowable amount of sodium ascorbate/erythorbate (560 ppm) may be needed for preservation of the cured meat color after the product is irradiated. An inoculated pack study on low level nitrite-nitrate ham (initial levels of 25 ppm NaNO₂ and 100 ppm NaNO₃) to determine the 12D radiation dose is in progress.

RADIATION RESISTANCE OF VEGETATIVE CELLS AT CRYOGENIC TEMPERATURES

With the exception of *Micrococcus radiodurans*, (1, 14) vegetative cells are generally more sensitive to radiation than sporeformers (14, 17, 18, 21, 27, 31, 33, 34, 41, 42). However, it is conceivable that at some cryogenic temperature certain non-sporeformers may equal, if not surpass, the radiation resistance of *C. botulinum* spores. With this in mind Anellis, et al. (4) recently screened the radiation resistance of the vegetative cells of 36 bacterial strains, comprising 19 species of 11 genera, at a radiation temperature of

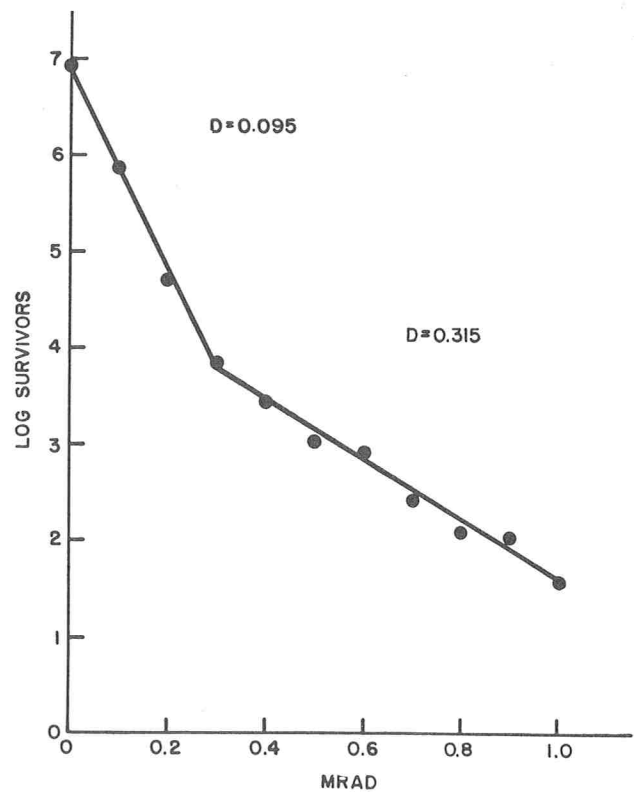


Figure 4. Resistance of *Streptococcus faecium* a21 to gamma rays at a radiation temperature of -80 ± 2 C. This curve represents 5.5 ml of cell suspension per tube, 2 tubes per dose, 2 plate counts per tube, average of 3 plates per dilution. Anellis, Berkowitz, and Kemper, 1973. Reproduced with permission of American Society for Microbiology.

-80 ± 2 C. Samples were irradiated in the range 0 to 2.7 Mrad in increments of 0.3 Mrad (Table 3). *M. radiodurans* was the most resistant, surviving 2.4 but not 2.7 Mrad. Three strains of *Streptococcus faecium* survived 1.5 but not 1.8 Mrad. Meat prepared for radappertization is normally heated to a center temperature of about 70 C (158 F) to inactivate the meat enzymes. Under these conditions the heat sensitive *M. radiodurans* (16) was not expected to present a radiation problem. However, due to the reported heat resistance of *S. faecium* (28) its survival as a function of dose and radiation temperature was studied in greater detail by Anellis et al (4). At all temperatures tested (5, -30 , -80 , -140 , and -196 C) two exponential survival curves were produced as shown in a typical plot (Fig. 4) of cells surviving various doses of irradiation at -80 ± 2 C. D values for the more resistant population (0.1%) were 2- to 3-fold higher than the D values of the initial slope. At an irradiation temperature of -30 C, strain α 21 had a D value of 0.11 and 0.24, respectively. The latter D value is higher than the D value (0.15 Mrad) for spores of *C. botulinum* 33A irradiated under essentially the same conditions (Anellis, et al., Unpublished data). Studies are in progress to determine the ability of *S. faecium* to survive when exposed to the total system (enzyme inactivation, freezing, and a 12D dose for *C. botulinum* spores) involved in the radiation processing of meats.

WHOLESOMENESS

One of the major goals in development of radappertized meats is still unresolved; that is, to provide proof convincing to the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) that irradiated foods are safe for unrestricted human consumption.

In 1963, the FDA approved radappertized bacon (4.5 to 5.6 Mrad, for unlimited human consumption. The bacon data together with data obtained from feeding of irradiated pork were again submitted to the FDA in 1966 in support of a ham petition. This time the reviewing agency raised doubts as to the safety of irradiated pork products. In their review of the ham petition, the FDA concluded that the safety of irradiated ham had not been established by the data (10). In 1968 the FDA revoked its previously granted approval of radappertized bacon.

In 1970, in cooperation with FDA, USDA, the Army Surgeon General's Advisory Committee on Nutrition and National Academy of Sciences-National Research Council Committees on Radiation Preservation of Foods and Radiation Sources, NLABS designed a new long term (3 years) feeding protocol

for irradiated beef to provide animal toxicity/carcinogenicity data on enzyme-inactivated frozen beef and the same beef sterilized by irradiation or heat (11). The data derived will be extrapolated to man. Beef, chosen as the test food instead of ham because of the nitrite, nitrate and nitrosamine uncertainties associated with ham, will be fed to rats and mice for up to 2 years and to dogs for 3 years. The five diet groups are shown in Table 4. The basal diets consisted of a semipurified diet for rodents (rats and mice) and a commercial ration for dogs. A semipurified diet (Table 5 was recommended by the FDA as the basal diet for the rodents because such diets permit control over the nutrient levels as well as minimizing the introduction of variables, both known and unknown, found in commercial rations. Another stated advantage was that rodents fed semipurified diets may be more sensitive to tumorigenic challenge than rodents fed commercial rations containing unidentified protective factors. A possible disadvantage was that semipurified diets have not been thoroughly tested and standardized for long-term multigeneration studies. Since the nutrition of the dog has not been as well defined as for the rodent, a commercial ration was selected for the dogs. The semipurified diet and the dry dog food will indicate the comparability and quality of the husbandry and management practices in the individual replicates. In addition to the basal diets, two other control diets (cooked, enzyme inactivated, frozen beef and thermally processed canned beef stored at ambient temperature) were included in the protocol. The thermally processed (F₀ = 5.8) beef represents a currently approved process. Of importance in this study is the comparison between the enzyme-inactivated frozen beef and the thermally processed beef on one hand and each of the irradiated beef groups on the other hand.

Dogs have progressed satisfactorily through the first year on the various diets, and the breeding of the dogs is now in progress. In the rodent studies difficulties have been experienced with the semipurified diet (11). It appears to be inadequate for growth and reproduction for mice and only marginally adequate for rats. In the F₀ generation 70 of 75 male mice fed the semipurified diet for 10-12 weeks died. Gross and histopathologic examinations revealed no apparent cause of death. The F₀ generation of rats fed the same diet progressed satisfactorily through growth and displayed normal reproductive performance. However, a large number of the first generation weanlings died. It should be noted that the rats fed 35% irradiated beef and 65% semipurified diet performed as well or better than the rats fed frozen or thermal beef plus semipurified diet. Rodent feeding tests were suspended in December 1972 pending resolution of

discussions with the FDA to change the semipurified diet. Dietary changes were made in the semipurified diet as follows: (a) Ten percent glucose was replaced with 2.0% dried whole beef liver, 3.0% dried yeast (Brewers), 2.5% commercial soybean containing 50% protein, and 2.5% alfalfa leaf meal; (b) increased the copper level in the salt mix fourfold; and (c) added selenium, fluoride, molybdenum, and chromium to yield final dietary concentrations of 0.1 ppm, 1.0 ppm, 1.0 ppm, and 1.0 ppm, respectively. In the event that these modifications would not correct the problem, additional groups of rodents were fed commercial rodent chow when the test was resumed in March 1973.

Measurements of concern include food consumption and efficiency, reproductive performance, longevity, hematology, ophthalmoscopic examination (rats and dogs), urology, and pathology. Periodic gross pathology will be conducted and, if lesions are observed, microscopic examinations will follow. Additional studies recommended by the FDA involve mutagenesis, teratogenesis, presence of pesticide residues and heavy metals, and toxicology of volatile radiolysis products in the beef. Mutagenicity studies will not be performed until there is agreement on the method of choice and its interpretation. Raw beef (not enzyme-inactivated), frozen enzyme inactivated beef, thermally processed beef and irradiated enzyme-inactivated beef are undergoing thorough chemical analyses for pesticide residues, heavy metals and organic radiolysis volatiles. All the data collected will be subjected to statistical treatment for significant differences between the diet groups. The entire feeding study, upon which the future of the radappertization of meats in the United States depends, is expected to be finished in the spring of 1976.

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SIGNIFICANCE OF BACTERIAL SPORES IN MILK^{1, 2}

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ABSTRACT

Sporeforming bacteria are present in practically all raw milk, but usually in small numbers if the milk is produced under modern sanitary conditions. There appears to be no relationship between total bacteria and spore counts, nor between total bacteria and the incidence of any given species of sporeforming organism. *Bacillus* species account for about 95% of the total sporeforming bacteria in milk, with *Clostridium* species comprising the remainder. In the United States, approximately 43% of the *Bacillus* organisms are *B. licheniformis* and 37% are *B. cereus*. Reports from some other countries indicate a predominance of *B. cereus*, or essentially a reversal in the prevalence of these two species. Spores of both these organisms survive low temperature pasteurization, some persisting through even the lower temperatures in the ultra-high range, and recent reports indicate that some sporeformers are psychrotrophic. *B. cereus* has been implicated in outbreaks of food-borne illness, but this is still controversial. Should *B. cereus* be proven capable of growing in the psychrotrophic range, and also responsible for food-borne illness, then the significance of spores in milk would take on added importance.

With the advent of bulk handling of milk in the Grade A and manufacturing milk segments of the dairy industry, types of bacteria in milk take on added importance. Milk is kept colder, and the normal raw milk flora is inhibited from growing as these bacteria once did when milk was handled in cans. One group of bacteria that has received considerable attention in recent years is the sporeformers. This paper reviews this work to assess the significance of bacterial spores in milk and milk products.

INCIDENCE OF BACTERIAL SPORES IN RAW MILK

Work was done in the early 1960's to determine the incidence and nature of the sporeformers in milk (9). Both Grade A and manufacturing grade milks were analyzed. The total bacteria counts and aerobic spore counts of the milk are given in Table 1. For convenience the milks are arranged in four classes (Class I to Class IV). Of the 176 samples of mixed herd milk, 19 had bacteria counts of < 50,000/ml, 36 had counts between 50,000 and 200,000/ml, 48 between 200,000 and 1,000,000/ml, and 73 between 1,000,000 and 5,000,000/ml. Since many of the milk

samples were received after a considerable lapse of time between production and analysis, the bacterial counts vary widely and do not represent accurately the bacterial quality of the milks at the point of production or at the local market.

The aerobic mesophilic spore counts varied from < 50 to 5,900/ml and averaged 400 to 760/ml for milks in the different categories. There were wide differences of individual counts within the various bacteria count classes, and no clear-cut relationship could be established between the total spore count and the total bacteria count. However, extremely high spore counts were always associated with high total bacterial counts.

Approximately 90% of the samples analyzed were positive for thermophilic sporeformers, but their numbers were extremely small. There was no relationship between the number of positive samples or the average thermophilic counts of the bacterial classes and the total bacterial counts. Of 297 sporeforming organisms which were isolated from the milk, 283 were identified conclusively as members of 10 different *Bacillus* species by the procedures outlined by Smith, et al. (15). These species are given in Table 2. Fourteen of the cultures were found to be members of the genus *Clostridium*, but the species were not identified. However, none of the *Clostridium* cultures were toxigenic according to the cultural characteristics outlined by Smith (14). Of the cultures belonging to the genus *Bacillus*, approximately 80% were members of two species, *B. licheniformis* (43.3%) and *B. cereus* (37.4%). The early work in the United States showed the sporeforming organisms in milk to be comprised of a rather high percentage of *B. subtilis*, but *B. licheniformis* was not represented (5, 6). The 1962 study revealed no *B. subtilis* among the sporeforming organisms isolated, but showed a high percentage of *B. licheniformis*. The difference between the findings may be attributed at least in part to the later development of more accurate methods for differentiating *B. subtilis* from *B. licheniformis*.

HEAT EFFECTS ON BACTERIAL SPORES

The suggestion has been made by several workers (1, 11, 12) that certain ultra-high temperature (UHT) treatments might stimulate germination and growth of bacterial spores, resulting in greater spoilage of

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²Presented at the 60th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Rochester, New York, August 13-16, 1973.

TABLE 1. STANDARD PLATE COUNTS AND AEROBIC SPORE COUNTS OF RAW MILK^{1, 2}

Class	SPC Range (per ml)	No. of samples analyzed	Avg. SPC (per ml)	Mesophilic spore counts	Thermophilic spore counts	
				Average (per ml)	No. of positive samples	Average (per ml)
I	<50,000	19	32,000	400	16	46
II	>50,000					
	<200,000	36	98,000	400	35	45
III	>200,000					
	<1,000,000	48	580,000	710	40	55
IV	>1,000,000					
	<5,000,000	73	2,300,000	760	60	41

¹Spore counts were determined after heating milk at 80 C for 10 min. Mesophilic counts were determined by a pour plate procedure; thermophilic counts by a Most Probable Number dilution tube technique.

²Data from 16th Int. Dairy Congress Proceedings, p. 295-304.1962.

UHT milk than of milk processed by conventional pasteurization. Conditions which appeared to be the result of such heat activation have been observed in actual practice (11). An investigation was completed in 1966 to explore the effects of several UHT treatments on germination and growth of the more common *Bacillus* species which are present in raw milk (8).

Results from that study with nine different *Bacillus* species subjected to UHT treatment of 104.5 C are in Table 3. The percentage destruction of the spores ranged from none for *B. laterosporus* to 50% for *B. pumilus*. The spores most commonly found in the raw milk supply, *B. licheniformis* and *B. cereus*, had destruction percentages of 16 and 25%, respectively. When this milk was incubated for 6 h at 35 C, the surviving spores of most of the species germinated and grew rapidly, with the vegetative cell count after 6 h ranging from 18.7 million/ml for *B. circulans* to 71 million/ml for *B. megaterium*. The spore counts declined as the vegetative cell count increased during incubation.

These data indicate that the UHT treatment at 104.5 C for approximately 1 sec does not destroy *Bacillus* spores effectively, and the spores which do survive are capable of germination and outgrowth when incubated at 35 C in the UHT milk.

Results obtained at 121 C are in Table 4. At this temperature, the destruction percentages for the spores were 99.7% or greater with all species except *B. licheniformis*, *B. laterosporus*, and *B. megaterium*. These species had destruction percentages of 97.7, 98.4, and 98.7% respectively.

Even though the numbers surviving were much lower than that of 104.5 C, surviving spores germinated and grew readily in the UHT milk heated at 121 C. For example, with *B. pumilus*, the spore count following heating was 62/ml, but after 6 h at 35 C, the number had increased to 1.5 million/ml. With *B. sphaericus*, with only 38 spores/ml surviv-

TABLE 2. ORGANISMS ISOLATED FROM RAW MILK SUPPLIES¹

Organism	Number isolated	% of Total
<i>Bacillus licheniformis</i> ²	129	43.3
<i>Bacillus cereus</i>	111	37.4
<i>Bacillus coagulans</i>	11	3.7
<i>Bacillus pumilus</i>	10	3.4
<i>Bacillus sphaericus</i>	6	2.0
<i>Bacillus cereus</i> var. <i>mycoides</i>	5	1.7
<i>Bacillus brevis</i>	4	1.3
<i>Bacillus laterosporus</i>	3	1.0
<i>Bacillus circulans</i>	2	0.7
<i>Bacillus stearothermophilus</i>	2	0.7
<i>Clostridium</i> (species unidentified)	14	4.7
Total	297	100.0

¹Data from 16th Int. Dairy Congress Proceedings, p. 295-304.1962.

²Differentiated from *Bacillus subtilis* by anaerobic growth in glucose broth and by colony characteristics on nutrient agar.

ing the 121 C temperature, there were 651,000 spores/ml (14 generations) after the incubation period. In contrast, *B. licheniformis* was less affected by this heat treatment than the other organisms, with the numbers increasing from 28,000/ml to 1.37 million (5.6 generations) during the 6 h incubation.

Results from a third heat treatment of 137.8 C (Table 5) indicate that the percentage destruction was 99.99% or greater with all nine *Bacillus* species. However, as with the intermediate heat treatment, the small number of survivors grew rapidly in the UHT milk, with total counts for *B. pumilus* and *B. megaterium* increasing from 8 to 15/ml to 85,000 and 518,000/ml, respectively, during 6 h at 35 C. A similar rate of growth was evident for all species except *B. licheniformis*, *B. cereus*, and *B. coagulans*, which showed total counts of 29, 5,800, and 1,200/ml, respectively, after the incubation period.

Using the data for outgrowth obtained in this study, the number of generations occurring during the incubation period was determined. Results are in Table 6. The number of generations for the species studied during incubation for 6 h at 35 C ranged

from 2.9 to 6.6, 5.6 to 15.5, and 2.3 to 15.1 in milks heated at 104.5, 121, and 137.8 C, respectively. The average number of generations for all species during the 6-h incubation was 5.2, 11.0, and 11.4 in the milk heated at 104.5, 121, and 137.8 C, respectively. For several of the species, *B. pumilus*, *B. cereus* var. *mycoides*, and *B. circulans*, the number of generations was about three or four times greater in the milks heated at 121 and 137.8 C than in the milk subjected to 104.5 C.

PERSISTENCE OF BACTERIAL SPORES IN DAIRY PRODUCTS

Presence of bacterial spores in raw milk, and the obvious heat resistance and activation of the species which are present, suggest a possible spore problem in manufactured dairy products. One of the most logical problem areas would be with soft-serve frozen desserts, since soft-serve frozen dessert mixes are usually pasteurized at a distant processing plant, delivered to the retail establishment, and held at refrigeration temperatures in the retail store until frozen for consumption. Opportunities for growth of contaminating organisms are extremely great under such conditions, since the refrigerated space is usually limited and serves as the storage area for other

food products sold at the soft-serve outlet. Doors to the refrigerated storage area are opened and closed frequently in most of these stores. As a result, the shelf-life of soft-serve mixes is quite variable.

Some processing plants have changed to UHT pasteurization in an attempt to improve the shelf-life of these products. This has not always been accomplished, and again reports from industry reveal that in some instances the shelf-life may actually be decreased in UHT-treated products. It has not been definitely established if the spoilage organisms survive the UHT treatment, or if they are post-UHT contaminants. If the microorganisms responsible for the short shelf-life are surviving the UHT conditions, then the most logical organisms involved are the heat-resistant sporeforming bacteria. Some evidence to support this supposition has been reported (8). If sporeformers are the predominant organisms in UHT milk, this could have particular significance in soft-serve frozen desserts, especially if the surviving organism were *B. cereus*, an etiologic agent of human gastroenteritis (10), and which is present in small numbers in most raw milk (9).

Therefore, a study was undertaken in 1971 to determine under pilot plant conditions the destructive

TABLE 3. EFFECT OF UHT TREATMENT AT 104.5 C (220 F) ON BACTERIAL SPORES^{1, 2}

Species	Spore count/ml		Approximate % destruction	UHT milk after 6 hr at 35 C	
	Before UHT	After UHT		Vegetative cell count/ml	Spore count/ml
<i>B. pumilus</i>	767,000	381,000	50	28,200,000	55,700
<i>B. coagulans</i>	547,000	301,000	45	28,000,000	87,400
<i>B. sphaericus</i>	813,000	450,000	45	43,500,000	68,300
<i>B. cereus</i> var. <i>mycoides</i>	803,000	583,000	27	30,300,000	483,000
<i>B. cereus</i>	870,000	657,000	25	34,600,000	497,000
<i>B. circulans</i>	1,530,000	1,240,000	19	18,700,000	1,050,000
<i>B. licheniformis</i>	1,240,000	1,050,000	16	22,300,000	363,000
<i>B. megaterium</i>	1,190,000	1,140,000	4	71,000,000	827,000
<i>B. laterosporus</i>	957,000	1,060,000	0	38,000,000	687,000

¹Average of three trials. Before addition of spores, the spore count of the raw milk was <60/ml.

²Data from J. Dairy Sci. 49:1367-1370. 1966.

TABLE 4. EFFECT OF UHT TREATMENT AT 121 C (250 F) ON BACTERIAL SPORES^{1, 2}

Species	Spore count/ml		Approximate % destruction	UHT milk after 6 hr at 35 C	
	Before UHT	After UHT		Vegetative cell count/ml	Spore count/ml
<i>B. licheniformis</i>	1,240,000	28,000	97.70	1,370,000	6,630
<i>B. cereus</i>	870,000	139	99.98	119,000	102
<i>B. coagulans</i>	547,000	171	99.97	111,000	158
<i>B. pumilus</i>	767,000	62	99.99	1,490,000	49
<i>B. sphaericus</i>	813,000	38	99.99	651,000	33
<i>B. cereus</i> var. <i>mycoides</i>	803,000	76	99.99	3,730,000	33
<i>B. laterosporus</i>	957,000	14,900	98.40	7,970,000	4,690
<i>B. megaterium</i>	1,190,000	15,600	98.70	29,700,000	10,100
<i>B. circulans</i>	1,530,000	147	99.99	285,000	184

¹Average of three trials. Before addition of spores, the spore count of the raw milk was less than 60/ml.

²Data from J. Dairy Sci. 49:1367-1370. 1966.

TABLE 5. EFFECT OF UHT TREATMENT AT 137.8 (280 F) ON BACTERIAL SPORES^{1, 2}

Species	Spore count/ml		Approximate % destruction	UHT milk after 6 hr at 35 C	
	Before UHT	After UHT		Vegetative cell count/ml	Spore count/ml
<i>B. licheniformis</i>	1,240,000	6	99.99	29	13
<i>B. cereus</i>	870,000	8	99.99	5,800	8
<i>B. coagulans</i>	547,000	3	99.99	1,200	9
<i>B. pumilus</i>	767,000	8	99.99	85,000	11
<i>B. sphaericus</i>	813,000	2	99.99	73,300	6
<i>B. cereus</i> var. <i>mycoides</i>	803,000	9	99.99	44,300	5
<i>B. laterosporus</i>	957,000	6	99.99	57,600	11
<i>B. megaterium</i>	1,190,000	15	99.99	518,000	3
<i>B. circulans</i>	1,530,000	3	99.99	35,100	17

¹Average of three trials. Before addition of spores, the spore count of the raw milk was <60/ml.

²Data from J. Dairy Sci. 49:1367-1370. 1966.

TABLE 6. EFFECT OF UHT TREATMENT ON GROWTH OF *Bacillus* SPORES IN MILK^{1, 2}

Species	No. of generations during 6 hr of incubation at 35 C after UHT treatment at		
	104.5 C	121 C	137.8 C
<i>B. licheniformis</i>	4.4	5.6	2.3
<i>B. cereus</i>	5.7	9.7	9.4
<i>B. coagulans</i>	6.5	9.3	8.6
<i>B. pumilus</i>	2.9	14.5	13.3
<i>B. sphaericus</i>	6.6	14.0	15.1
<i>B. cereus</i> var. <i>mycoides</i>	5.7	15.5	12.2
<i>B. laterosporus</i>	5.1	9.0	13.1
<i>B. megaterium</i>	5.9	10.8	15.0
<i>B. circulans</i>	3.9	10.8	13.4
Average no. of generations	5.2	11.0	11.4

¹No. generations = $3.3 \log_{10} \frac{\text{final no.}}{\text{initial no.}}$

²Data from J. Dairy Sci. 49:1367-1370. 1966.

effects of UHT treatments on spores of *B. cereus* in frozen dessert mixes, and to evaluate the post-pasteurization shelf-life of these products under variable conditions of refrigerated storage (7).

Spores of *B. cereus* were used in pilot plant studies with UHT-treated frozen dessert mixes. Skim-milk powder of low thermophilic count was used in the mix formulation, and the mix was subjected to 137.7 C for 3 sec before inoculation with *B. cereus* spores. The bacteriological quality of these mixes pasteurized at 104.5 C and 137.7 C after incorporation of more than one million spores/ml of *B. cereus* during refrigerated storage is presented in Table 7.

Heating at 104.5 C destroyed only 12.5% of the spores; whereas 99.5% were destroyed by heating at 137.7 C. However, in the mix stored at 4.4 C, numbers remained almost constant for 6 weeks and then began a gradual decline, and the product did not show visual spoilage even after 8 weeks of storage. At 10 C, the product processed at 104.5 C showed visual evidence of spoilage and large numbers of *B.*

TABLE 7. BACTERIOLOGICAL QUALITY OF *Bacillus cereus*-CONTAINING SOFT-SERVE FROZEN DESSERT MIXES PASTEURIZED AT 104.5 AND 137.7 FOR 3 SECONDS DURING STORAGE AT 4.4, 10, AND 15 C^{1, 2}

Storage time	Plate counts after storage at:					
	4.4 C		10 C		15 C	
	Pasteurization temperature		Pasteurization temperature		Pasteurization temperature	
	104.5 C	137.7 C	104.5 C	137.7 C	104.5 C	127.7 C
0 Time	1.4×10^6	6.6×10^3	9.9×10^5	1.6×10^4	1.4×10^6	6.6×10^3
1 Week	1.4×10^6	3.1×10^3	4.9×10^7	2.7×10^7	2.2×10^7	1.2×10^8
2 Weeks	1.6×10^6	4.0×10^3	6.2×10^7	6.0×10^7	$1.6 \times 10^{8**}$	$1.5 \times 10^{8**}$
3 Weeks	1.6×10^6	2.7×10^3	3.0×10^7	3.1×10^7		
4 Weeks	1.3×10^6	2.6×10^3	$8.2 \times 10^{6**}$	2.0×10^7		
5 Weeks	1.2×10^6	3.0×10^3		$9.8 \times 10^{6**}$		
6 Weeks	1.2×10^6	4.8×10^3				
7 Weeks	8.8×10^5	3.1×10^3				
8 Weeks	7.6×10^5	3.2×10^3				

**Denotes curdled or proteolyzed samples.

¹The control mix to which no spores were added was pasteurized at 137.7 C; no organisms were evident immediately after pasteurization, and there were fewer than 20 organisms/ml throughout the storage period. In the UHT-treated samples stored at 4.4 and 15 C, the initial spore inoculum was 1.6×10^6 /ml; in the samples stored at 10 C, the initial spore inoculum was 1.05×10^6 /ml.

²Data from J. Milk Food Technol. 34:256-259. 1971.

cereus after 4 weeks of storage, and at 15 C after only 2 weeks of storage. After 5 weeks and 2 weeks at 10 C and 15 C, respectively, visual evidence of spoilage and large numbers of bacteria were noted in product processed at 137.7 C, indicating that storage at 10 C or above of softserve mixes containing *B. cereus* spores could possibly create both spoilage and public health problems.

Data obtained emphasize the importance of low-temperature storage even with UHT-treated products. Fortunately, many of the sporeforming bacteria in the normal flora of raw milk are in the vegetative state, and are easily destroyed by conventional pasteurization. Therefore, no such problems are likely to arise in a mix processing operation if the ingredients used in commercial formulations do not supply large numbers of spores, and if strict sanitation procedures are followed.

CONCLUSIONS

Bacterial spores are present in most raw milk, and any laxity on the part of producers and processors can instigate problems, especially in manufactured dairy products. Recently, several workers have reported the isolation of sporeformers which grow at cold temperatures (2, 3, 4, 13). Such evidence emphasizes even more the significance of bacterial spores in milk and milk products. However, careful sanitary procedures coupled with proper pasteurization and low temperature storage of the finished product should preclude any problems with sporeforming organisms.

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A Research Note

FATE OF COLIFORM BACTERIA IN BUTTERMILK¹

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(Received for publication August 7, 1973)

ABSTRACT

Coliform bacteria in buttermilk decreased on storage at 4 C and 37 C. Counts decreased at 20 C when the initial inoculum was low but increased when it was high. The change in the number of coliform bacteria was not correlated with either the total acid or acetic acid concentrations, or with their kinetics. In 10% skim-milk lactic and acetic acid had complementary inhibitory effects on coliform bacteria, except when the acetic acid concentration was below 10%, then the interaction between the two acids was not consistent.

The behavior of coliform bacteria in *Streptococcus lactis*-based dairy products is somewhat variable. In most instances coliform bacteria counts decrease during product storage (4, 5, 7, 9) but increases in counts on storage also have been reported (4, 6, 8). The present report describes the nature of the variability of coliform bacteria counts in buttermilk.

METHODS

Buttermilk obtained from a commercial dairy had been prepared as follows: 1.5% fat pasteurized milk with 0.2% added salt was ripened with *Streptococcus lactis* and aroma bacteria for 15-20 h at 18-20 C. The coagulum was then stirred and poured into polyethylene bags. Samples were incubated in the laboratory at 4, 20, or 37 C. Acidity (1), pH, and acetic acid content were determined; the last by gas chromatography on Carbowax 20M of the acidified supernatant obtained on centrifuging the buttermilk at $5000 \times g$ for 5 min.

For tests with added acids, coliform bacteria were isolated on violet red bile agar from a buttermilk sample and transferred to 10% skimmilk. After 24 h aliquots were transferred to 250 ml containing 10% skim-milk with various amounts of lactic or acetic acid and incubated at 4, 20, and 37 C. Coliform bacteria were counted on violet red bile agar (2) and, in some instances, checked against plate count agar.

RESULTS AND DISCUSSION

Mean changes in total acidity and acetic acid concentration in buttermilk at various storage temperatures are shown in Fig. 1. Both parameters show an increase with time, the increase being greater at higher storage temperatures. The level of titratable acidity is similar to that observed previously in other *Streptococcus lactis*-based products (6, 9). However,

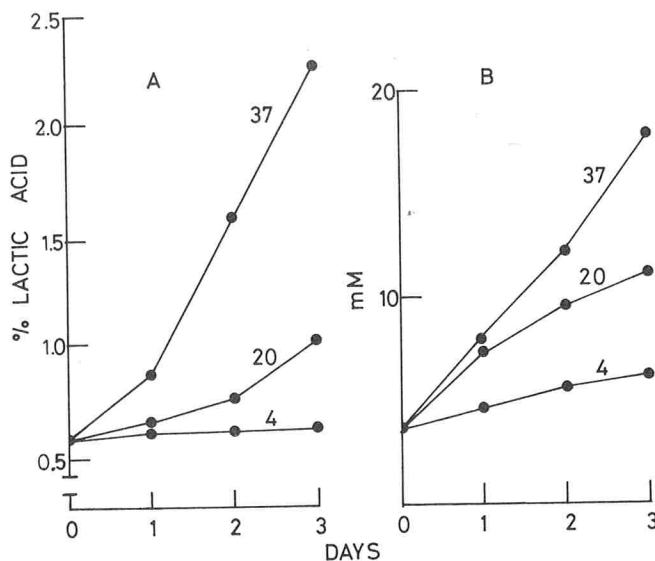


Figure 1. Mean increase in total acidity (as % lactic acid) A, and in acetic acid, B, with time of storage at several temperatures. The storage temperature is indicated on the curve. A is the mean of six duplicate samples and B the mean of four duplicate samples.

TABLE 1. EFFECT OF STORAGE TEMPERATURE AND INITIAL VRB PLATE COUNT ON 90% DEATH TIME OF COLIFORM BACTERIA IN BUTTERMILK. (RESULTS ARE MEANS OF TWO DUPLICATE DETERMINATIONS)

Initial coliform count	Storage temperature		
	4 C	20 C	37 C
	----- (hours) -----		
9×10^3	19.9	26.3	12.0
3×10^4	14.2	15.4	14.0
4×10^5	98.7	P ¹	13.9
7×10^5	79.4	P	13.5

¹Proliferation, 90% death time indeterminable.

Streptococcus lactis alone would not be expected to produce more than 1% acidity. Considerable counts were observed on Rogosa agar (10^4 - 10^6 /g) and on potato dextrose agar at pH 3.5 (10^3 - 10^5 /g) and the presence of these organisms may account for the high titratable acidity on storage at higher temperatures.

Table 1 shows the time for 90% death of the coliform bacteria at the various temperatures of storage. Incubation at 4 and 37 C resulted in death of the organisms at all initial coliform levels, the rate of death being greater at 37 C. Storage at 20 C ap-

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²Supply Corps, Israel Defence Forces.

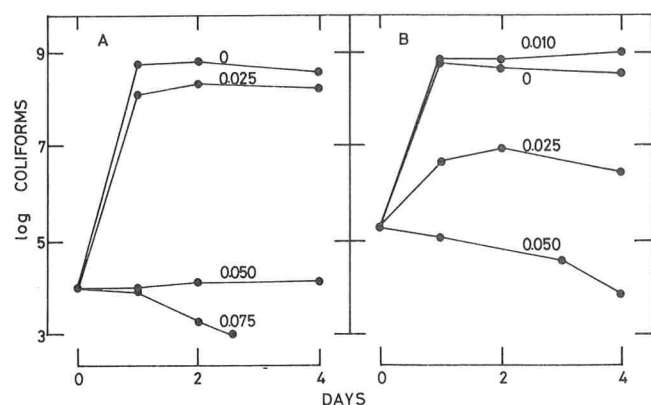


Figure 2. Effect of time and lactic acid (A) and acetic acid (B) concentration (M) on coliform bacteria counts on storage at 20 C.

peared to be the most favorable temperature for the coliform bacteria, a high initial inoculum resulting in proliferation. We have previously reported a similar rise in coliforms where high initial inocula were present in another *Streptococcus lactis*-based cultured milk product (6). Changes in the coliform population were not correlated with the total acidity, with the acetic acid concentration, or with their rate of change.

In an attempt to clarify the effect of lactic and acetic acid on the coliform bacteria, a series of experiments were done with 10% skim milk containing various concentrations of lactic and acetic acids (Fig. 2); effects were parallel at 20 and 37 C. Lactic acid concentration became inhibitory to growth of coliform bacteria at 0.05-0.065 M and acetic acid became inhibitory at 0.04-0.05 M. The concentration at which lactic acid inhibited coliform bacteria in skim milk was much lower than the titratable acidity found in buttermilk. Combinations of these two acids led to complementary effects, except where <10% of the total acid was acetic acid, when inconsistent

stimulatory or inhibitory effects were observed. A metabolic mechanism controlling the lactic and acetic acid metabolism of microorganisms has been reported (3), but the nature of this interaction is not clear. However, neither total acidity nor acetic acid concentration appeared to be the factors controlling death of coliform bacteria in this system.

Results of the present study reinforce the earlier conclusions (6, 9) that the use of coliform bacteria as a sanitary indicator is valid in fermented products only when carried out on the fresh product.

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EDWARD C. SNYDER RETIRES

Edward C. Snyder of Northwood Townhouses, Geneva, New York, has taken early retirement as Quality Control Manager of Comstock Foods Division, Borden, Inc., at Rushville, after eleven years with the Company. Ed was formerly connected with the Milk Industry in Central New York, in many capacities; a Butter maker, Field Manager, and Receiving Plant Manager. He and Mrs. Snyder owned and

operated the Cazenovia Dairy at Cazenovia, N. Y. prior to WW II.

Ed, a former President of the Central New York Sanitarians, is a member of the IAMFES, NYSAMS, National IFT, and Western N. Y. section-IFT.

Ed and Mrs. Snyder are retiring to their Florida home at 1906 Ohio St. T. E., Bradenton 33507.

EFFECT OF MEAT PARTICLE SIZE AND CASING DIAMETER ON SUMMER SAUSAGE PROPERTIES DURING DRYING¹

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ABSTRACT

Drying characteristics were evaluated for summer sausages (50% beef, 50% pork) prepared with three meat particle sizes obtained through grinding variations. Increases of chemical components (protein, fat, ash, salt, lactic acid) during 45 days of drying were dependent on the rate of moisture removal from sausages. Summer sausage produced with a 9-mm grinder plate for the pork and a 6-mm plate for beef (9-6 grinding combination) had a 34% shrinkage at 45 days, whereas sausages of a 3-6 and a 6-6 grind combination had shrinkages of 37% and 40%, respectively. The rate of moisture removal for an all beef summer sausage was lower for larger diameter sausage when 52, 62, and 73 mm sizes were compared. Moisture content of the outer one-third radius portion of the sausages was 5 to 7% lower than the moisture content of the inner two-thirds radius portion from 5 days through 45 days of drying. Both types of summer sausages (beef-pork and all beef) having greater than 1.2 kg/cm² of shearing force were generally of poor eating and slicing quality because of the dried fibrous condition of the meat.

Fermented and nonfermented dry sausages are generally classified as semidry, medium dry, and dry according to moisture content. A wide variety of products is possible within any one classification because of variables in meat formulation, particle size, seasoning, extent of fermentation, intensity of smoke flavor, processing temperature, and types of casing (9). Fermented sausages possess good keeping quality because of low pH and high salt content, and in dry varieties, a low moisture content.

Use of commercial starter cultures has significantly reduced fermentation time, incidences of off-flavor development, and casing "explosions" resulting from growth of undesirable bacteria (6, 7, 8). Lyophilized starter cultures were introduced in 1957 and frozen concentrates were available in 1968 (4, 5, 7). Acton et al. (1) reported that fermentation of summer sausage at either 22, 30, or 37 C did not significantly affect product flavor even though less lactic acid was produced at the lower temperature.

The rate of drying is a critical factor in producing

an acceptable product. The drying period required to achieve a desired endpoint moisture level is dependent on temperature, humidity, and air turnover rate (13). Drying too rapidly causes case hardening and prevents adequate drying of the sausage interior.

This study was done to evaluate the effects of meat particle size and casing diameter on the rate of drying, composition, and shear values for fermented-dried summer sausage.

MATERIALS AND METHODS

This study was conducted in two parts. In Experiment 1, the effect of meat particle size on summer sausage drying was studied using a beef-pork meat mixture (Table 1). The meat particle sizes chosen for investigation represent the range of grinder plate sizes normally used in the final grinding for dry sausage products. In Experiment 2, an all beef summer sausage

TABLE 1. SUMMER SAUSAGE INGREDIENTS

Ingredient	Quantity	
	Experiment 1	Experiment 2
Meats:		
lean beef, boneless	2.27 kg	4.54 kg
lean pork trimmings	2.27 kg	
Cure:		
sodium nitrite		0.35 g
sodium nitrate		0.70 g
sodium erythorbate		2.12 g
salt		113.4 g
Seasonings:		
ground black pepper		8.5 g
ground white pepper		8.5 g
ground mustard		8.5 g
sucrose		22.6 g
Starter materials:		
Lactacel MC (diluted) ^a		23.0 ml
dextrose		45.4 g

^aA frozen concentrate of *Pediococcus cerevisiae* and *Lactobacillus plantarum* from Merck & Co., Rahway, New Jersey. A culture suspension is prepared by diluting 6 oz concentrate with 18 oz distilled water.

(Table 1) was prepared to examine the effect of sausage diameter on drying properties. Individual batch quantities of curing agents, seasonings, and starter culture materials were the same for both experiments. The two summer sausage product styles, "Farmer" (Experiment 1) and "Beef" (Experiment 2), were similar in ingredients to those in commercial use.

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TABLE 2. COMPOSITION¹ AND pH OF INITIAL MEATS AND SUMMER SAUSAGE DURING DRYING AS AFFECTED BY MEAT PARTICLE SIZE (EXPERIMENT 1)

Meat item	Particle size	Moisture %	Protein %	Fat %	Ash %	NaCl %	Lactic acid %	pH
Lean beef, boneless	—	66.7 ± 0.1	19.1 ± 0.2	12.6 ± 0.3	0.9 ± 0.1	Trace	0.0	5.8
Pork trimmings	—	60.3 ± 0.2	16.2 ± 0.2	23.4 ± 0.2	0.8 ± 0.1	Trace	0.0	5.9
Summer sausage:								
0 days drying	3-6	59.8 ± 0.1	19.2 ± 0.2	18.4 ± 0.2	3.0 ± 0.1	2.4 ± 0.1	1.3 ± 0.2	4.3
	6-6	58.4 ± 0.1	18.6 ± 0.1	18.7 ± 0.2	—	2.4 ± 0.1	1.3 ± 0.1	4.3
	9-6	58.7 ± 0.2	19.0 ± 0.1	17.2 ± 0.3	—	2.4 ± 0.1	1.3 ± 0.1	4.3
15 days drying	3-6	42.2 ± 0.2	33.1 ± 0.2	22.6 ± 0.3	3.6 ± 0.2	3.3 ± 0.1	2.2 ± 0.1	4.3
	6-6	40.9 ± 0.3	29.4 ± 0.1	26.1 ± 0.2	—	3.4 ± 0.1	2.2 ± 0.1	4.3
	9-6	41.1 ± 0.1	32.7 ± 0.1	24.3 ± 0.2	—	3.4 ± 0.1	2.2 ± 0.2	4.3
30 days drying	3-6	32.4 ± 0.2	33.5 ± 0.1	29.3 ± 0.2	4.1 ± 0.2	3.8 ± 0.2	2.4 ± 0.1	4.4
	6-6	31.6 ± 0.4	35.8 ± 0.2	31.5 ± 0.3	—	3.9 ± 0.1	2.6 ± 0.1	4.4
	9-6	35.2 ± 0.1	34.8 ± 0.2	28.6 ± 0.1	—	3.9 ± 0.2	2.4 ± 0.2	4.4
45 days drying	3-6	28.6 ± 0.1	35.7 ± 0.2	32.5 ± 0.1	4.2 ± 0.2	4.0 ± 0.2	2.5 ± 0.2	4.4
	6-6	27.8 ± 0.1	36.1 ± 0.3	34.2 ± 0.2	—	3.9 ± 0.1	2.6 ± 0.2	4.4
	9-6	31.3 ± 0.3	35.1 ± 0.1	31.8 ± 0.3	—	4.0 ± 0.2	2.5 ± 0.1	4.4

¹Means followed by standard error of the mean.

Source and portions of meat

Fresh boneless beef (chucks) and pork trimmings were obtained from the state inspected Meats Laboratory of the Animal Science Department at Clemson University. Boneless beef was ground twice through a 6-mm plate with thorough mixing between grinds.

Pork trimmings were coarsely ground once through a 9-mm plate, mixed and divided into three portions. One portion was reground once with a 3-mm plate, the second portion with a 6-mm plate, and the third portion was reground through the 9-mm plate.

Experiment 1 - Sausage fermentation and processing

Pork trimmings from each grinding procedure (3-, 6-, and 9-mm plates) were individually combined with equal quantities of the ground beef to provide the following particle size or treatment designations: (a) 3-6; (b) 6-6; and (c) 9-6. The first number refers to the smallest orifice diameter in millimeters of the plate used in grinding the pork trimmings. The second number was the smallest orifice diameter in millimeters of the plate used for grinding the boneless beef.

The beef and pork meats were blended in a Hobart 4346 Mixer-Grinder equipped with two mixing arm paddles. The curing agents, seasonings, and dextrose were blended into the meat mixture before addition of the starter culture. A mixed starter of *Pediococcus cerevisiae* and *Lactobacillus plantarum* (LACTACEL MC) was added to a level of approximately 6.5×10^7 cells/g meat. The initial mix temperature was approximately 2 C and increased to approximately 8 C during 10 min of blending at 29 rpm. Three replicate sausage batches per particle size combination were prepared.

Each sausage preparation was stuffed into 52-mm diameter D.S. fibrous casings (Union Carbide). The sausage chubs, each weighing approximately 600 g were hung in a fermentation chamber maintained at 38 C and 95% relative humidity. The sausages were held to ferment for 24 h.

Following fermentation the sausages were initially heated (no smoke) at 82 C for 2 h and then at 93 C until an internal temperature of 62 C was obtained (3.0-3.5 h total). The product was cooled to 16 C with a cold water spray and placed in a 7.5 ± 2.0 C drying room having 10-15 air changes/h.

The air relative humidity ranged from 88-92%. Sausage chubs were removed for analysis at 0, 5, 10, 15, 30, and 45 days of drying.

Experiment 2 - Sausage fermentation and processing.

Methods described in Experiment 1 were modified as follows: (a) meat for sausage preparation was all boneless beef; (b) after inoculation with the starter culture, the sausage mix was stuffed into either 52-, 62-, or 73-mm diameter D.S. fibrous casings; (c) the fermentation was terminated after 18 h. The heat processing method and drying room conditions were the same as those used in Experiment 1. Three replicate sausage batches per casing diameter were prepared.

Sample chubs of each replicate and sausage diameter were removed for analysis at 0, 5, 10, 15, 20, 30, and 45 days of drying.

Analysis of sausage composition

Percentages of moisture, fat, protein, ash, and salt were determined for sausage samples at each interval of drying. Moisture was determined by the AOAC (2) method. Ether extractables (Soxhlet) were used to calculate percent fat. The Kjeldahl nitrogen method following AOAC (2) was used for protein determinations. Percent ash was determined by the AOAC (2) method. The salt content, expressed as NaCl, was measured with QUANTAB Chloride Titrators following the procedure of the AOAC (3) and Vander Werf and Free (11).

TABLE 3. CORRELATION COEFFICIENTS BETWEEN CHEMICAL COMPONENTS AND SAUSAGE MOISTURE CONTENT DURING DRYING OF SUMMER SAUSAGES

Chemical component	Correlation coefficient, r	
	Experiment 1	Experiment 2
Protein, %	-0.97**	-0.99**
Fat, %	-0.97**	-0.99**
Ash, %	— ¹	-0.94**
NaCl, %	-0.99**	-0.94**
Lactic acid, %	-0.98**	-0.99**

**Highly significant ($p \leq 0.01$)

¹Insufficient data for correlation analysis.

All compositional analyses were done in duplicate.

The percent moisture in two locations of 52-mm, 62-mm and 72-mm diameter sausages (Experiment 2) was determined during the 45-day drying period. After measuring the diameter of each sausage at the sampling intervals, the outer one-third radius portion and the inner two-thirds radius portion were separately collected, ground, and analyzed for moisture (2). Samples were taken from a 3.0-cm thick slice at the center of the sausage chub.

pH and lactic acid determinations

Duplicate 10-g samples of meat from each replicate and drying interval were blended for 60 sec with 100-ml quantities of distilled water in an Osterizer. The pH values of homogenates were recorded with a pH meter. All sample slurries were then titrated with 0.1 N NaOH to an endpoint pH of 8.30. The mEq of alkali required to raise the pH to 8.30 for a fresh sausage sample before fermentation were deducted from the total mEq required for titration of samples after fermentation. Developed acidity was assumed to result from lactic acid production. The mEq of NaOH were converted and expressed as percent lactic acid.

Physical determinations

The percent weight loss or "shrink" of summer sausage chubs was determined at each stage of drying. Two chubs from each replicate (6 per treatment) were selected after heat processing (0 day drying) for weight recording.

Shear forces for slices of the summer sausage samples were measured using an Allo-Kramer Shear Press equipped with a 1362-kg ring. The press was used with a downstroke of 30 sec at range 300. Meat slices were 3 mm in thickness. The shearing force was calculated as kg force/g sample/cm² of surface area exposed to the shear blades. A surface area expression was included since the slices were of variable diameter during the drying phase.

RESULTS AND DISCUSSION

Experiment 1

During the 45-day drying period, increases in protein, fat, ash, salt, and lactic acid contents (Table 2) of all sausages were significantly correlated (Table

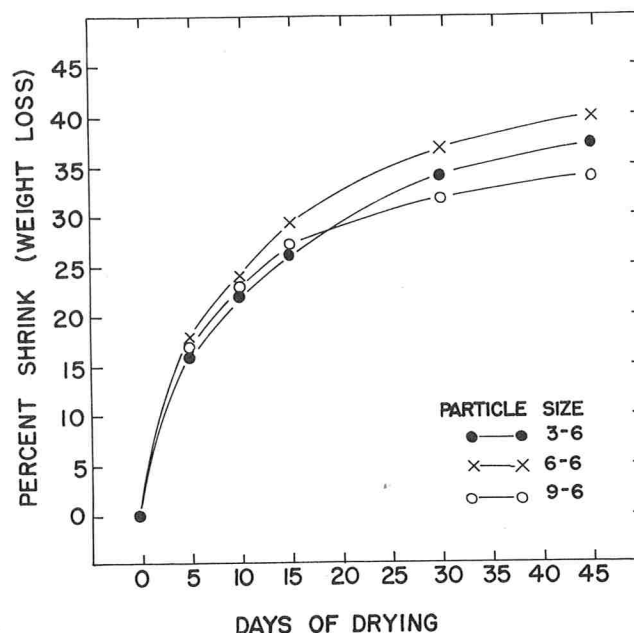


Figure 1. Percent shrinkage during drying of summer sausages varying in meat particle size (Experiment 1).

3) with the decrease in moisture level. The fermented sausage pH of 4.3 did not decrease as the lactic acid became concentrated on drying. At 30 days, the pH had increased by 0.1 pH unit. Wardlaw et al. (12) suggested that the increase of pH during sausage drying is due to the accumulation of basic nonprotein nitrogen compounds.

The amount of moisture removed followed previously estimated shrinkage schedules listed by Wilson (13). The influence of meat particle size on the rate of moisture removal was not apparent until 30 days of drying. At the 30-day interval (Table 2)

TABLE 4. COMPOSITION¹ AND pH OF INITIAL MEAT AND SUMMER SAUSAGE DURING DRYING AS AFFECTED BY SAUSAGE DIAMETER (EXPERIMENT 2)

Meat item	Casing diameter, mm	Moisture %	Protein %	Fat %	Ash %	NaCl %	Lactic acid %	pH	
Lean beef, boneless	—	65.8 ± 0.1	18.2 ± 0.1	14.3 ± 0.2	0.9 ± 0.1	Trace	0.0	5.8	
Summer sausage:	0 days drying	52	60.8 ± 0.2	22.6 ± 0.2	13.9 ± 0.3	3.5 ± 0.1	2.4 ± 0.1	0.9 ± 0.1	4.6
		62	60.5 ± 0.2	21.1 ± 0.2	14.8 ± 0.2	3.4 ± 0.2	2.3 ± 0.1	0.9 ± 0.1	4.6
		73	60.3 ± 0.1	21.3 ± 0.1	13.8 ± 0.3	3.5 ± 0.1	2.4 ± 0.1	0.9 ± 0.2	4.6
	15 days drying	52	42.7 ± 0.3	30.6 ± 0.1	22.2 ± 0.3	4.6 ± 0.2	2.9 ± 0.1	1.4 ± 0.2	4.7
		62	45.5 ± 0.3	28.8 ± 0.3	21.2 ± 0.2	4.6 ± 0.1	3.0 ± 0.1	1.4 ± 0.1	4.6
		73	45.8 ± 0.1	28.4 ± 0.1	20.4 ± 0.2	4.5 ± 0.1	3.0 ± 0.1	1.4 ± 0.1	4.6
	30 days drying	52	31.1 ± 0.2	36.1 ± 0.2	27.4 ± 0.3	5.7 ± 0.2	3.6 ± 0.2	1.9 ± 0.2	4.9
		62	34.8 ± 0.2	34.1 ± 0.2	26.6 ± 0.3	5.6 ± 0.2	3.8 ± 0.1	1.8 ± 0.1	4.9
		73	37.5 ± 0.4	33.0 ± 0.2	25.3 ± 0.2	5.5 ± 0.2	3.8 ± 0.1	1.7 ± 0.3	4.9
45 days drying	52	28.9 ± 0.2	37.3 ± 0.3	29.4 ± 0.2	6.1 ± 0.3	4.1 ± 0.2	2.0 ± 0.2	4.9	
	62	32.2 ± 0.3	36.4 ± 0.2	29.1 ± 0.1	6.0 ± 0.2	4.2 ± 0.2	2.0 ± 0.2	4.9	
	73	34.1 ± 0.4	34.2 ± 0.2	28.7 ± 0.2	6.0 ± 0.2	4.2 ± 0.2	1.9 ± 0.2	4.9	

¹Means followed by standard error of the mean.

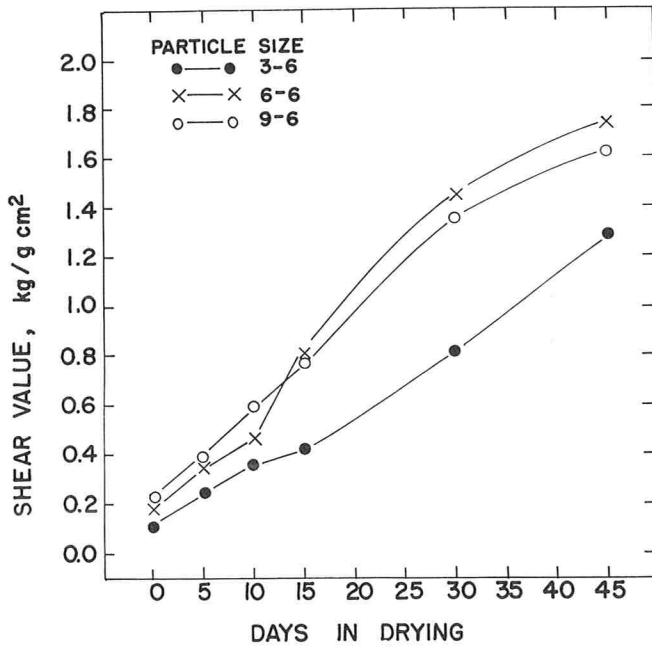


Figure 2. Shear values during drying of summer sausages varying in meat particle size (Experiment 1).

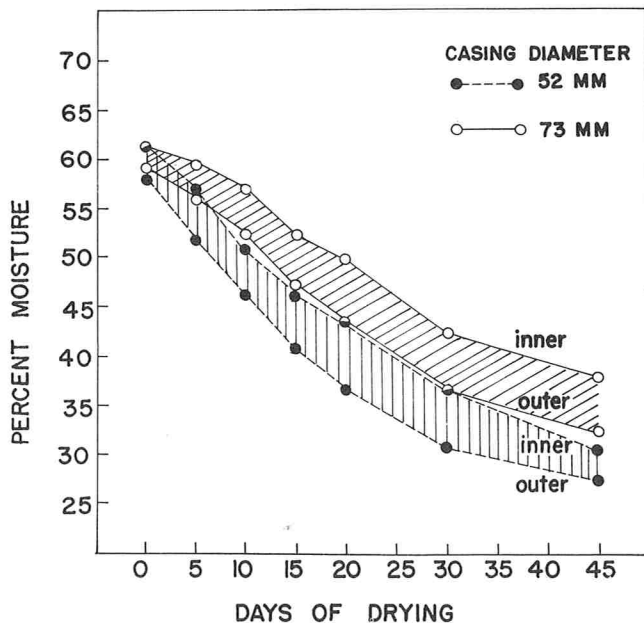


Figure 3. Comparison of percent moisture of the inner and outer portions of summer sausages during drying (Experiment 2).

and 45-day interval the moisture content of the 3-6 and 6-6 samples was significantly lower than the moisture content for the larger 9-6 sausage samples. Examination of the shrinkage curves (Fig. 1) shows that at 15 days, no major differences in chub weight losses occurred. However, at 30 and 45 days, the shrinkage of the 6-6 ground sausage ranged from 3% to 7% higher than that of the other sausages.

No case-hardening was observed at the surface of

the sausage chubs. It is possible that the particle sizes of meat may "pack" differently on mixing and stuffing. The larger 9 mm meat particles would tend to lose moisture rapidly from their surface, hindering moisture release from the particle interior. With the 3 mm meat particles, the finer matrix formation of the small particles may act to spread around the larger meat particles (50% were 6 mm) forming a barrier for moisture release after an initial rapid loss (Fig. 1). Further study using meat(s) of a single grind will be necessary to determine the exact nature governing the rate of moisture release.

Using typical "dryness" classifications (13), the semidry stage (20-25% shrink) was attained for all sausages between 10-15 days. The medium dry stage (30-50% shrink) was attained for all samples between 15-20 days of drying. The dry stage (35-40% shrink) was reached by 30 days for the 6-6 sausages and by 35 days for the 3-6 sausages. However, further drying (after 25 days) of the 9-6 summer sausage did not produce a shrink over 35% at the 45 day period, the longest interval in this study.

The two summer sausages having the larger particle sizes also exhibited the greater shear values (Fig. 2). No apparent relationship existed between sausage moisture content and sample shear force. Although no taste panels were conducted, the authors observed that meat slices of the 6-6 and 9-6 sausages were beyond reasonable eating quality for dried sausage after 30 days due to the tough, fibrous characteristic of the dehydrated meat. The chubs were also extremely difficult to machine slice.

Experiment 2

The diameters stated in this section refer only to the initial stuffed sausage diameters. During 45 days of drying, the diameters decreased from 52, 62, and 73 mm to 37, 48, and 58 mm, respectively.

Chemical changes in each sausage diameter group (Table 4) were dependent on and correlated (Table 3) with the rate of decrease in moisture content. As expected, the rate of moisture loss was slower as the sausage casing diameter increased. At 30 days, a 6.4% difference in moisture content between the 52-mm and 73-mm diameter sausages occurred. The initial sausage pH of 4.6 after 18 h of fermentation increased to pH 4.9 and 5.0 at 30 and 45 days of drying, respectively. These values are within the range of 4.5 to 5.4 generally reported for summer sausage at the semidry stage. (9, 10).

Diffusion of moisture from the sausage interior to the surface was not hindered by case-hardening. Moisture analysis of the exterior (outer 1/3 radius) and interior (inner 2/3 radius) portions of the drying sausage of 52- and 73-mm diameter are shown in Fig.

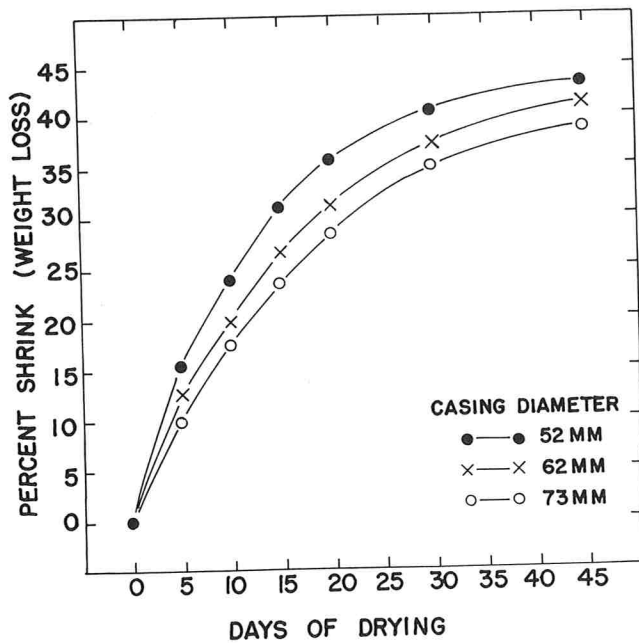


Figure 4. Percent shrinkage during drying of summer sausages varying in diameter (Experiment 2).

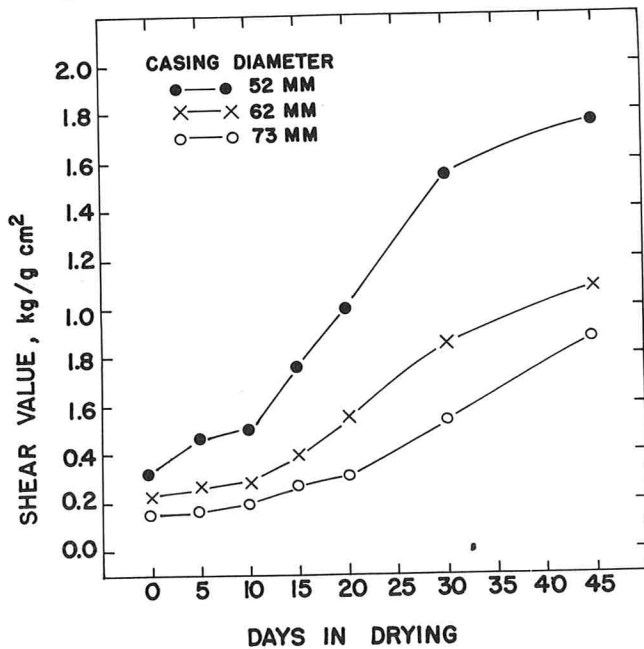


Figure 5. Shear values during drying of summer sausages varying in diameter (Experiment 2).

3. In most instances, the location differences did not exceed 7% moisture. Similar location differences were observed for sausages of the 62-mm diameter, the values being intermediate to those shown in Fig. 3. Shrinkage curves for each diameter group are given in Fig. 4. The maximum shrinkage of 40-45% was attained between 30 to 40 days for the 52 and 62 mm sausages. Sausages having shrinkages above 40% are considered "fully dried" (13).

Sausage hardness, as determined by shear force

values, are shown in Fig. 5. Comparison of the 52-mm diameter curve to that of the 6-6 sausage in Experiment 1 (Fig. 2) shows almost the same rate of hardness development. However, the slower rate of dehydration of the 62 and 72 mm sausages is reflected in a slower rate of hardening in comparison to the 52-mm diameter sausage.

From shear results of both experiments, it appears that at a shear value of approximately 1.2 kg/g/cm², the sausage becomes undesirable in eating quality due to the dried fibrous condition. It may be possible to alter the dried textural characteristics, however, by increasing fat levels over that used for the initial meats in this study. Further study of textural characteristics using instrumental evaluation and taste panels are needed.

ACKNOWLEDGMENTS

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REPORT OF THE IAMFES MEMBERSHIP COMMITTEE 1972-1973

The Membership Committee has just completed its first full year of activity. The first few months of the past year was spent in preparation before we could get into any active organized solicitation of members.

EXECUTIVE BOARD MEETING

At the Executive Board meeting in Milwaukee (August, 1972) the membership committee was given permission to spend up to \$1,200.00 to publish a membership brochure. We had submitted a text to the Executive Board for this purpose. A copy of the text was given to every member of the board for their revision and corrections. A deadline was set for them to return it so that it could all be compiled into one manuscript and sent to Dr. Elmer Marth for editing. This was completed in time so that the Executive Board at its meeting in Chicago in December gave final approval to the lay-out and text.

Incidentally, it should be mentioned that the text and the photographs which we selected to be printed in this brochure were given to Mr. P. J. Skulborstad who in turn gave them to Mr. Don Sobkoviak and Mr. Robert Muth in the Babson Bros. art department. They took this material home and spent their evenings doing the art work and compiling the highly professional brochure which the Executive Board unanimously approved at the Chicago meeting. If it had not been for the help of these men in the Babson Bros. organization we would not have been able to publish this brochure within the budget. It was February before we could actually mail any of the brochures, but it is my understanding that since that time 6000 have been mailed to the membership chairmen of the various affiliates.

NEW AFFILIATES

In April of this year (1973) I met with Walter Wilson, H. L. "Red" Thomasson, Harold Barnum, and K. G. Weckel in Denver to attend the first annual meeting of the revived Inter-Mountain affiliate at the University of Colorado in Fort Collins. This meeting was largely the result of work done by Harold Barnum and Dr. Dean of the University of Wyoming. In my opinion it was a huge success and I believe that we can look forward to an annual meeting of the revived Inter-

Mountain Association and the membership will naturally increase.

Just recently Dave Cleveland of Oklahoma City agreed to become the Membership chairman for Oklahoma. Oklahoma is in the same position that the Inter-Mountain Association was last year. However he has 40 people with whom he has arranged to attend an organizational meeting anytime that Earl Wright, H. L. Thomasson or the Membership Chairman can attend to help them re-organize and write their new constitution.

The same condition exists in Arizona. However we would be a little farther along in Arizona if it were not for the fact that at three different scheduled meetings someone of importance to the association was either ill or out of the State. We are still attempting to set a date that will be satisfactory with everyone. Mr. Wilson has agreed to attend this meeting. We have had considerable correspondence from Mexico City and I hope that before the annual meeting next year we will have an affiliate in Mexico.

SUMMARY

The activities of the Membership Committee have resulted in: (a) one new or revived affiliate; (b) three prospective affiliates; (c) a membership brochure of which 6000 have been put into the hands of the local affiliate membership chairmen; we had 9 IAMFES membership chairmen last year; this year we have 21 plus "Red" Thomasson; (d) we have received applications from: 136 new direct members, and 169 new affiliate members for a total of 303 new members.

We hope to do better next year. From our experience this year we have found that it is easier and more profitable to solicit members from outside the organization rather than try to revive members who have dropped out. The food industry and the environmental health field offer us a much greater opportunity to get younger and more active members and that is where we hope to concentrate our efforts in 1973-1974.

HAROLD HEISKELL
Chairman

FROZEN CONCENTRATED CULTURES OF LACTIC STARTER BACTERIA. A REVIEW¹

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ABSTRACT

Concentrated starter cultures are now used routinely in many food fermentations. Recent literature concerning preparation and performance of such cultures is reviewed. Batch procedures are preferred over continuous culture procedures for preparing concentrated cultures. Most attention has been focused on bacteria used to manufacture cultured milk products. Performances of concentrated cultures of lactic streptococci, leuconostocs, lactobacilli, and thermophilic streptococci in preparing cultured dairy products have compared favorably to those of traditionally prepared milk cultures. Storage in liquid nitrogen appears to be the best method for long term holding of concentrated cultures.

INTRODUCTION

Concentrated starter cultures consist of starter culture bacteria which have been grown under closely controlled conditions in a liquid medium, concentrated into a smaller volume, and then placed in frozen storage. Such procedures include several variations from those customarily used for starter preparation and may alter markedly the characteristics of the culture. The growth medium must contain all the nutrients needed for optimum growth of the bacteria and the resulting cells must contain the necessary complement of enzymes and biological activity to insure proper performance of the concentrated cultures when used to manufacture cultured foods. In addition, the composition of the growth medium should not prevent or hinder the harvesting of the cells. While it is not always possible, a growth medium with a composition similar to that of the food to be bioprocessed would help insure that the concentrated cultures contain adequate biological activity. The freezing medium must provide protection to the cells to minimize damage resulting from freezing.

METHODS FOR GROWING CELL CROPS

At the present time concentrated starters can best be prepared by batch procedures. Performance of such starters in manufacturing various cultured dairy products has been compared to that of traditional milk starter cultures. (1, 9, 10, 27). The types of bacteria

included in these studies were: lactic streptococci (1), leuconostocs (9, 10), lactobacilli and thermophilic streptococci (27). In all instances concentrated cultures produced cultured products equal to or better than those manufactured using traditionally prepared milk cultures.

Equipment and growth requirements for continuous production of bacterial cells have received considerable attention in the literature (5, 14, 16, 17, 19, 20, 21, 22, 26). Most of these reports have dealt only with cell production and not actual preparation and evaluation of concentrated cultures. Keogh (17) reported that a single strain concentrate from a continuous culture performed as well as a conventional milk starter in the manufacture of Cheddar cheese. Lloyd and Pont (22) found that concentrated cultures prepared in a similar manner produced cheeses which ripened normally and were of comparable quality to those prepared using normal bulk starters. However, in some trials acid was produced more slowly in cheese milk by the concentrates than by conventional cultures. Two of the seven strains of lactic streptococci used in their study degenerated during the continuous culture process. This was attributed to increased phage susceptibility or loss of enzymatic activity. Another problem which might occur in continuous culturing is the development of undesirable mutants (24).

CULTURE EVALUATION

There are several ways in which the cell crops for concentrates should be evaluated. While the number of cells produced is important, measurement of the cells' ability to produce the desired changes during food bioprocessing is more important. To obtain the fullest advantage from using culture concentrates, organisms in the concentrate must possess metabolic activity at least comparable to that of organisms in starters prepared by the conventional method. The metabolic activity of the culture must be maintained throughout its preparation, shipment, and storage so that the cultured product will possess the characteristics desired.

¹Paper No. 4218 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina.

LACTIC STREPTOCOCCI

In producing culture concentrates it is economically important to obtain the largest yield of cells possible that possess maximum biological activity. The population of lactic streptococci can be greatly increased by controlling the pH of the growth medium at a favorable level (2, 4, 5, 22, 25, 26). Growth of the streptococci at pH 6.0-6.5 appear to favor production of maximum cell numbers. In the study by Peebles et al. (25) cultures were grown in a broth medium containing 2% tryptone, 1% yeast extract, 2.5% lactose, and 2.5% glucose contained in fermentors equipped with automatic pH control units. Sodium hydroxide was used as the neutralizer. The highest population was obtained when the culture was grown at pH 6.0 which was about 15 times that obtained with no pH control. Time required to reach the maximum population at pH 6.0 was less than that required at either pH 5.5, 6.5, or 7.0. These results indicate pH 6.0 to be the optimum for both the rate and total amount of growth. Similar results were obtained for other cultures.

The neutralizer used for automatic pH control also influences the maximum obtainable population of the lactic streptococci (22, 25). Populations obtained for three single strain lactic streptococci when grown at pH 6.0 using ammonium hydroxide as the neutralizer were 1.5 to 2.5 times greater than when sodium hydroxide was the neutralizer. The maximum population was also reached in a shorter period for each culture neutralized with ammonium hydroxide. In evaluating the activity of cultures prepared with both neutralizers, cells of strain ML1 prepared with sodium hydroxide produced acid at a rate comparable to that of a conventional milk culture of the same strain. Acid production by cells of culture ML1 prepared with ammonium hydroxide lagged behind that of milk-grown cells. This decreased rate of acid production was, however, regained after one subculture of the concentrate in milk. This deleterious effect was not observed for all cultures prepared using ammonium hydroxide as the neutralizer. In examining the cause for the lower activity of the cells of ML1 prepared with ammonium hydroxide as the neutralizer, we found that the proteinase activity of such cells was very low. On subculture of concentrated cells, the proteinase activity was largely regained. Lloyd and Pont (22) also observed a similar effect when using NH_4OH as the neutralizer.

The number of cells of lactic streptococci produced at pH 6.0 in 12 h was almost as great as that present after 24 h (25). Nevertheless, cells were metabolically very active between the 12-24 h period. The rate of neutralizer addition decreased after the maximum

population was reached but did not completely stop until about the 24th hour. To determine whether the cells were affected by longer or shorter incubation in the fermentor, cells were harvested after 18 and 24 h. Acid production by equivalent numbers of cells in milk from the two concentrates had essentially the same acid producing ability as did a conventional milk culture. Similar findings were observed for all cultures evaluated. On the other hand, Bergere (3) indicated that cells of lactic streptococci should be harvested just before reaching the stationary phase to insure optimum activity of the concentrated cultures.

Since production of cells appeared to stop before nutrients were exhausted or before acid production stopped in the fermentor, we were interested in determining the causes for such a limitation in cell production. Keen (15) and Linklater and Griffin (19) reported that cultures of lactic streptococci grown in a medium with the pH automatically maintained at a level favorable for growth were inhibited because carbon dioxide was depleted from the medium. Cogan (5), however, has shown that sparging the medium with carbon dioxide during growth provided no benefit for the cultures. Lactic acid or lactate salts have also been suggested as the factor which limits growth of lactic streptococci (4, 26). Spent media from culture growth in our studies (12, 25) contained compounds that retarded growth of a new inoculum. By paper chromatography and bioautography several zones of inhibitory material were found to be present in the spent medium (12). Of three zones showing inhibition, one was most consistently inhibitory. The material in this zone was identified as leucine. However, L-leucine added to the broth medium was not inhibitory. Subsequent study revealed that D-leucine was present and was the inhibitory component. Apparently, this compound is a metabolite of the streptococci and acts as an auto-inhibitor.

Related studies (13) involving milk cultures have indicated that other metabolites also act as auto-inhibitors of the streptococci. Measurable quantities of hydrogen peroxide are produced by the lactic streptococci during growth. Addition of active catalase prevented accumulation of hydrogen peroxide and permitted faster acid production. All cultures of lactic streptococci, which have been tested, produced acid more rapidly in the presence of catalase which eliminates the accumulation of auto-inhibitory levels of hydrogen peroxide. This has been found for laboratory strains as well as for commercial cultures of streptococci used for Cheddar cheese manufacture. Aeration of the culture accentuated peroxide formation. Keen (16) also has shown that the streptococci produce hydrogen peroxide when grown at pH 5.45

to 6.35. Sufficient peroxide was formed to retard growth of the cultures.

It has been our experience that not all starter cultures can be successfully prepared as concentrates. Evaluation of a concentrate prepared from a commercial starter revealed a markedly reduced ability to produce acid in milk by the concentrate. Activity was regained during subculture so that by the third subculture, acid production was essentially equal to that of the milk culture. On the other hand, a different commercial starter culture was found to be equally as active when prepared as a concentrate or when maintained routinely in milk. The reduced activity by these concentrated cultures may have resulted from alterations in cellular metabolic capabilities or to other factors such as an imbalance among strains of streptococci in the culture. Stadhouders et al. (31) suggested that procedures used for making concentrated cultures might alter the strain balance of the culture.

Some starter cultures are maintained as multiple strain starters and we questioned the consequences of preparing such starters as concentrates particularly in a medium different from milk in which they are normally maintained. The preparation of concentrates was found to have a variable effect on the balance of strains as determined by strain dominance, using bacteriophage susceptibility as a test system (8). For example, commercial culture A maintained in milk produced acid more rapidly than did the culture prepared as a concentrate. However, when both preparations were challenged by the specific bacteriophage, the culture maintained in milk had a reduced ability to produce acid, whereas the concentrate was unaffected. These data indicated that the strains that were resistant to bacteriophage, predominated in the concentrated culture. In starter culture B, opposite results were obtained and the concentrate contained strains susceptible to the bacteriophage active against this culture. With starter culture C, cells from a concentrated starter were found to produce acid faster than those from regular milk cultures. However, the bacteriophage had a somewhat more deleterious effect on the concentrate than it had on the milk culture. These data suggested that possibly some antagonistic interactions among strains in the original culture existed, since their elimination or partial elimination during concentrate preparation improved their acid-producing ability. Obviously, in preparation of concentrated starters, attention must be given to the possible alteration of strain balance as a result of their having been grown in a medium other than milk. Otherwise, the ability of concentrates to perform properly in milk may be unexpectedly decreased.

LEUCONOSTOCS

The importance of the composition of the medium on the resultant quality of concentrates was also observed for *Leuconostoc citrovorum* (9, 10). Since this culture is used to manufacture of dressing for cottage cheese, its ability to produce diacetyl and flavor compounds is important to the quality of the cottage cheese. The concentrates prepared from a growth medium, in which no sodium citrate was present, had essentially no ability to produce diacetyl even though numbers of cells were quite high. When the concentrate was subcultured in media containing citrate, the ability of the culture to produce diacetyl was regained. If concentrates were prepared from broth media containing sodium citrate, then the cells possessed adequate capability for diacetyl production. Comparisons of the ability of *L. citrovorum* to produce diacetyl after growth in milk with the concentrate of the same culture grown in a medium containing sodium citrate, indicate that the concentrates possessed greater capability for production of diacetyl. Furthermore, the concentrates produced essentially the same volatile components as did cultures grown in milk (11).

LACTOBACILLI

The medium used to prepare concentrates can have effects on the survival of the cells after freezing and thawing. It has been observed that among strains of *Lactobacillus bulgaricus* certain strains are particularly susceptible to effects of freezing even in liquid nitrogen (28). Cell viability as well as the ability of the concentrate to produce acid is greatly reduced. Efforts to protect the cells against damage by the use of cryoprotective agents were unsuccessful. Survival of cells grown in one medium had a very satisfactory survival rate. Considering the importance of the cell membrane to cellular integrity and stresses, it was suspected that the Tween 80 in this medium was the effective ingredient. By adding Tween 80 to the medium which normally produced labile cells, it was found that the cells resisted freezing quite successfully. The concentration of Tween 80 required for maximum resistance varied. Subsequent testing has shown that the oleate portion of Tween 80 is the important ingredient that confers resistance to freezing when cells are grown in this medium.

STORAGE AND SHIPMENT

Concentrated cultures must be stable to storage to allow shipment to processing plants. Storage of concentrated cultures in liquid nitrogen (-196°C) has

been recommended as the best means to preserve the starters (6, 10, 21, 25, 28, 29). Stadhouders et al. (30), however stated that storage of concentrated cultures of lactic streptococci at -196 C offered no special advantage over storage at -37 C when 7.5% lactose was added as cryo-protective agent. The extent of their storage period was 6 weeks. Several groups have reported the suitability of storing frozen concentrates in the range of -20 to -40 C for limited times (1, 3, 18, 22, 30, 31). On the other hand Moss and Speck (23) have shown that freezing and storing cells of *S. lactis* at -20 C resulted in considerable death plus metabolic injury. The injury was expressed as the inability of a portion of the surviving cells to grow on a restricted agar medium. Furthermore, lactic streptococci frozen and stored at -20 C lost a considerable amount of proteinase activity whereas those stored at -196 C did not (6, 7, 29). Glycerol has been shown to be useful as a cryoprotective agent when the cultures were stored at -20 C (18) and at -40 C (31) and Lamprech and Foster (18) indicated that concentrated cultures survived freezing better if frozen at a pH near neutrality (pH 6.6-7.0). Peebles et al. (25) stored concentrates of lactic streptococci (suspended in milk) for as long as 231 days with no loss of viability or activity. This appears to be the best method for long term storage of concentrated cultures.

Our present knowledge of bacterial starters has allowed the preparation of successful starter culture concentrates. But, we need to improve our knowledge of the metabolism of starter cultures. Particularly, information is needed on effects of stresses on cellular viability and activity. As such information is obtained more efficient and productive uses will be made of starter culture concentrates.

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INTERIM REPORT OF THE COMMITTEE ON APPLIED LABORATORY METHODS, 1972-1973

The Applied Laboratory Methods (ALM) Committee of the International Association has continued to be active in the affairs of the Association. The two subcommittees of this committee have provided assistance and consultation in the following areas: (a) Developed and conducted collaborative and/or comparative studies on established, modified, and new laboratory methods which have been and/or will be published in the *IAMFES Journal*. The data from these studies assisted in the preparation of the 13th edition of *Standard Methods for the Examination of Dairy Products* (1972) and will also be of inestimable value during preparation of the 14th edition of *Standard Methods*. (b) Provided assistance to the National Mastitis Council (NMC) and the National Mastitis Council Research Committee. (c) Continued to encourage uniformity of laboratory methods for examination of dairy products and other foods. Such uniform utilization of recommended methods should provide more meaning to food microbiological standards for foods. (d) Continued to encourage development of criteria for certification of laboratory apparatus, reagents, media, and materials in all disciplines concerned with the examination of foods and interpretation of results relative to protection of the public. (e) Provided liaison, through representation on other IAMFES Committees.

The 1970-1972 Report of the ALM Committee projected reactivation of the ALM Subcommittee on Laboratory Methods for the Examination of Foods during 1972-1974. Although a Chairman has not been selected for this committee, this committee should be activated before the 1975 meeting and plans formulated for fulfillment of projected committee assignments.

An Intersociety Council has been designated to develop protocols for preparation of the 14th edition of *Standard Methods for the Examination of Dairy Products*. The IAMFES representative to the APHA Intersociety Council is not a member of this ALM committee; apparently, designation of IAMFES representatives to APHA and other laboratory related organizations by the executive board of IAMFES does not involve consideration or consultation by the ALM committee.

Although representation of the ALM has not occurred on the Intersociety Council, at least six members of this IAMFES Committee will be directly involved in the preparation of the 14th edition of *Standard Methods*. During the next 2 years, it is anticipated that comparative and/or collaborative studies will be conducted, completed, and possibly published, to update laboratory methods which have low levels of precision.

At least two members of the ALM committee have been invited and are participating with another Intersociety Council responsible for preparation of a compendium of sampling and microbiological laboratory methods for the examination of foods. Activation of the ALM Subcommittee on Laboratory Methods for the Examination of Foods should provide necessary assistance for finalization of this Compendium on food laboratory methods.

A. Richard Brazis,
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REPORT OF THE SUBCOMMITTEE ON LABORATORY METHODS FOR THE EXAMINATION OF MILK AND MILK PRODUCTS

The activities of the subcommittee during the past year have centered around two laboratory studies. One was the comparison of 45 vs. 50 C for pouring plates; the other was an evaluation of the need for phosphate buffer in the dilution blanks.

The main reason for the first study was to demonstrate the importance of properly controlling agar temperature in pouring plates, in view of the sensitivity of many psychrotrophic bacteria to heat. *Standard Methods*, 12th Edition, recommends that melted medium be cooled promptly to about 45 C and then held at 44-46 C. Temperature control, according to SMEDP recommendations, should be by inserting a thermometer in a flask of water and comparing this temperature to a similar flask of agar. The heat transfer of water is faster than of agar and it is conceivable that if the procedures are followed, the final pouring temperature could be considerably higher than 44-46 C.

This report will not cover the computational procedures of this or the subsequent experiment. Briefly, the results showed a highly significant reduction of counts at 50 C. There was no difference in reproducibility between the two temperatures although there were differences in reproducibility between analysts. This has been consistently observed in other studies by the Subcommittee. We would suggest from this study that a more precise method of temperature control be developed. Perhaps the simplest would be to insert a thermometer in a spare flask of agar rather than in a flask of water.

The second of our studies involved the necessity of adding phosphate buffer to the milk dilution blanks. The literature on this subject does not indicate the reasons for using phosphate for milk dilutions; actually the diluted milk contributes more phosphate than that added to the diluting water. The only reference cited by *Standard Methods* to use of phosphate is a paper by Butterfield in 1932. He studied recoveries of bacteria from river water in variously treated waters and found higher numbers when phosphate was used. No studies were ever reported on the necessity of phosphate for milk plating. Our studies showed slightly increased counts in blanks without phosphate. These differences were not significant when analysis of variance was used but the differences were significant by other statistical means. This discrepancy was due to highly significant interactions encountered in the analysis of variance. At any rate the dilutions without phosphate were at least as high in counts as those with phosphate. As of this writing we have not decided on any recommendations arising from this study. Possibly phosphate could be omitted from the dilution water for the first dilution of the sample, but could be retained in dilution blanks where additional dilutions of sample(s) are necessary.

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Donald I. Thompson, State Laboratory of Hygiene, 437 Henry Mall, Madison, Wisconsin 53706.

Donald Pusch, Technical Quality Assurance, The Pillsbury Company, Minneapolis, Minnesota.

REPORT OF THE SUBCOMMITTEE ON LABORATORY METHODS
FOR THE EXAMINATION OF WATER AND OTHER
ENVIRONMENTAL SAMPLES

Acting Chairman, Gene Ronald made initial communication with all subcommittee members on January 4, 1973. Such consultation was necessary to remind the subcommittee members of the specific changes of this ALM subcommittee and to solicit their thoughts and suggestions concerning a proposal project study.

The replies from the members indicated a strong interest and desire to actively support and participate in a project selected by the majority of the members. This project study concerns: a comparison of coliform growth characteristics in lactose and/or lauryl tryptose broth; samples will be collected from shellfish waters and non-halogenated treated water supplies. The format for conducting this study has been distributed to the members. By deleting additional variables from the original format, it is possible that this project study will be completed and submitted for publication prior to the Biennial Report of the ALM committee in 1974.

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Gene Ronald, *Acting Chairman*, State Hygienic Laboratory, Des Moines Branch, Des Moines, Iowa 50309.

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John C. Hoff, Environmental Control Administration, Northwestern Water Hygiene Laboratory, Gig Harbor, Washington 98335.

Frank Busta, Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55101.

Kenneth Whaley, Sanitary Bacteriology Laboratory, State Health Department, Nashville, Tennessee 37219.

REPORT OF THE COMMITTEE ON FOOD
EQUIPMENT SANITARY STANDARDS—
1972-1973

The IAMFES Committee on Food Equipment Sanitary Standards, known hereafter as the Committee, is charged with the responsibility of cooperating with other interested health organizations and related industries in, the formulation of sanitary standards and educational materials for fabrication, installation, and operation of food equipment and to present to the membership those standards and educational materials which the Committee recommends be endorsed by the Association.

The purpose of this cooperative program is to aid industry in improving the design, construction, and installation of equipment so that it will lead to easy cleaning and proper functioning when placed into service in food establishments. It is the Committee's further purpose to cooperate with industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nationwide acceptance of such equipment.

The following report will outline the Committee's activities during the past year in working with two health and industry organizations (National Sanitation Foundation's Joint Committee on Food Equipment Standards and the National Automatic Merchandising Health-Industry Council) and progress in meeting its purposes and objectives. It is expected these organizations will be the two groups that the Committee will work with during the coming year.

NATIONAL SANITATION FOUNDATION (NSF)

The Committee was represented at the 1973 meeting of the National Sanitation Foundation's Joint Committee on Food Equipment Standards, where action was taken on several proposals; and before the meeting, the Committee reviewed and submitted comments on each draft of these proposals. Since the meeting, the Committee has also reviewed and submitted comments on proposed changes to standards.

The Foundation staff is to be commended for setting aside the first half-day for a meeting of the public health representatives for them to review with the Foundation matters of mutual interest. This enabled the public health group to develop a productive and a uniform approach to agenda items and to expedite activities during the meeting of the Joint Committee.

Basic Criteria C-2 for special devices and equipment

Item 1.01 of C-2 specifies that a component part or device which is covered under existing NSF standards or criteria shall be evaluated against those requirements. In answer to a question from the Foundation staff as to applicability of these requirements to refrigerated condiment dispensers, it was the unanimous agreement of the public health representatives that refrigerated condiment dispensers shall comply with the requirements of NSF Standard No. 7 in terms of temperature and operating times, as well as with the sanitary requirements of C-2.

The Foundation staff advised that a number of current NSF standards contain references to the ASA National Plumbing Code and because said document has been withdrawn and copies are not available, such a reference is not appropriate. The public health representatives concurred unanimously with the Foundation staff that the plumbing code of the Building Officials and Code Administrators International be utilized in future references in C-2 and all NSF standards. The Foundation, in concurrence with the public health group, has made plans to use the metric system as well as the English measurement system in the future printing of standards and criteria.

The subject of coin operated office coffee dispensing equipment was reviewed with the representatives; and they recommended, and the Foundation staff concurred, that the current requirements of the National Automatic Merchandising Association for special coin operated devices be reviewed and consideration be given to preparing a similar document as an NSF standard or criteria.

Standard No. 1—Soda fountain and luncheonette equipment

The three-year review of Standard No. 1 has been complet-

ed; and this extensive review, along with recommendations from this Committee and others, have been reviewed and recommended for approval by the Joint Committee. Copies of the final draft of the proposed amendments to Standard No. 1, as recommended for approval by the Joint Committee, may be obtained from the Foundation.

Standard No. 2—Food service equipment

The subject of detachable drainboards was discussed at the request of a sink manufacturer. After considerable discussion and reviewing potential problems, public health representatives reaffirmed their previous position that detachable drainboards should not be permitted. They also reaffirmed their previous position of limiting use of woodtop tables to bakery equipment, since there was no additional information provided to justify removal of the limitation.

Because of the interest among some members of the public health group, particularly this Committee, to develop specific criteria for enclosed food transport cabinets or carts to improve cleanability was reviewed by the Joint Committee. After much discussion, the majority of the public health representatives recommended that the proposed revision, including the definition of enclosed transport cabinets, be recommended for adoption as revised with the exception of the item determining the performance, which will be studied further by a task committee. It is hoped that this task committee would also consider and recommend that joints and seams be sealed with some material other than one of the synthetic compounds to preclude the entrance of moisture rather than limiting them to being closed.

The Foundation staff presented for consideration of representatives the problem of colored containers which are intended for use both for refuse storage and food storage. According to the group, these containers are fabricated under the provisions of NSF Standard Nos. 2 and 21 for Food Service Equipment and Thermoplastic Refuse Containers, and comply with both sets of requirements. Therefore, they should be permitted to bear the wording, "Listed for Food or Trash Containers."

Standard No. 7—Walk-in and reach-in refrigeration

The Foundation staff presented a request by a state health agency that remote mounted thermometers be provided on the outside of all refrigerators and storage freezers listed under the provisions of NSF Standard No. 7. This matter was thoroughly discussed by the public health representatives, including the advantages and disadvantages of this type of thermometer and mounting, and the majority of the representatives felt that a revision to accommodate this request was not warranted at this time. However, it is hoped that this very reasonable request will be implemented by at least the next revision of Standard No. 7.

The subject of drains in walk-in refrigerators and storage freezers was reviewed. Advantages and disadvantages of requiring or prohibiting drains in walk-in boxes were discussed; and regardless of urgent recommendations from the IAMFES representative to fabricate such refrigeration units to permit installation of floor drains, where desired, the majority of public health representatives was in favor of continuing the present requirements of Standard No. 7 eliminating drains in prefabricated walk-in boxes.

The public health representatives reviewed a request from a manufacturer of prefabricated walk-in refrigerators for a revision in NSF Standard No. 7 relative to eliminating the requirement for sealed (gasketed) joints and seams. It was the group consensus that the current provisions of the standard were adequate as they related to joints and seams, but ad-

ditional attention should be given to the provisions and requirements of installation. Furthermore, a standards task committee should be convened to consider development of a provision in the standard wherein the manufacturers would be required to provide detailed instructions covering the proper installation of their walk-ins.

Standard No. 18—Food and beverage dispensing equipment manual

The request of a manufacturer to include a cup stop on his dispensing equipment activation mechanism was presented to the public health group. It was the consensus of the group that the cup stop in question would, in fact, minimize a more serious potential public health problem; i.e., immersion of the dispensing head into the glass. However, the Foundation should establish parameters for shape and size to minimize contamination of the cup and to facilitate cleanability and maintenance of the stop and surrounding area.

Standard No. 20—Bulk milk dispensing equipment

Standard No. 20, with a few significant changes such as provisions for single service containers and tubing, were recommended for approval after an interpretation from the PHS/FDA representative as not in conflict with FDA.

Standard No. 25—Vending machines for food and beverages

The Foundation staff presented a preliminary report of the Standards Task Committee on Atmospheric Vented Devices for Vending and Dispensing Equipment which is recommended deletion of the requirement for atmospheric vented devices in both NSF Standard Nos. 18 and 25 due to lack of epidemiological evidence that there was a significant hazard from copper poisoning, and that those devices now available would not continue to function over an extended period. Further, the Task Committee recommended that the previous requirements relating to double check valves be reinstated. As requested by public health representatives present, the Foundation staff has obtained additional epidemiological data from the Center for Disease Control in Atlanta, Georgia before proceeding with any revisions in the referenced standards and this has been disseminated to members of the Joint Committee for review and comment. The IAMFES has recommended that this matter which has been under consideration by NSF and NAMA be studied very carefully before making a final decision as to a definite plan of action.

Future plans

A complete revision of Standard No. 2 is being considered by a standards task committee; and a special task committee will be charged to review the problem of adequate guards for smorgasbords, buffets, and cafeterias, as well as utensil storage equipment, from the public health viewpoint, and to submit appropriate recommendations. Furthermore, special task committees have been charged to review the need for a drain cock, plug, and drain to facilitate flushing and draining of water bath compartments in water cooled beverage dispensing equipment; to develop test procedures for foam cups and containers; to develop proposed standards for retail food store refrigerators, and to develop proposed standards for floor matting used in food establishments.

The aforementioned proposals should be ready for review by this Committee during the coming year. Another proposal to develop standards for carpeting for food service operations has not been implemented, according to the Foundation staff, due to lack of industry interest.

NATIONAL AUTOMATIC MERCHANDISING ASSOCIATION (NAMA)

The National Automatic Merchandising Association's Auto-

matic Merchandising Health-Industry Council (AMHIC) held its 17th annual meeting during October 1972, and this Association and other public health organizations and the affected industries were represented and participated in AMHIC's discussion.

The morning of the first day was reserved solely for a meeting of the public health representatives and was used by them to discuss and clarify their view on public health objectives and policies to be followed in their work with the entire membership of the AMHIC. The Chairman of the IAMFES Food Equipment Committee was re-elected Chairman of the Public Health Group and also served as Co-Chairman of AMHIC during 1972-1973.

Coin-operated special dispensers

According to the AMHIC Committee on Special Dispensers, this proposed Standard for Coin-Operated Special Dispensers is intended to establish reasonable and effective guidelines for the sanitary design and construction of a type of food and beverage dispensing device which was not in existence when the original U.S. Public Health Service Ordinance and Code, *The Vending of Food and Beverages*, was published.

Small dispensers of the so-called "office type," when coin-operated, meet the definition of "vending machine" which has been widely adopted in state and local vending sanitation laws, regulations, and ordinances. However, their size, types of products dispensed, capacity, and other operational aspects make it impractical and unnecessary for such dispensers to meet all of the traditional vending machine design and construction requirements.

This Standard provides a basis for public health evaluation of such dispensers, as required by many state and local statutes, under the NAMA Special Dispensers Evaluation Program. Further, it provides the manufacturers of such equipment relevant design and construction guidelines for sanitation purposes.

The proposed coin-operated Special Dispenser Standard was approved unanimously as amended at the 1972 meeting of AMHIC, and copies of this new Standard may be obtained from the National Automatic Merchandising Association.

There has been tremendous interest in the development of such a Standard during the past few years, and it is indicative by the last report from the NAMA headquarters that one special dispenser has already been evaluated and found to be in compliance with this new Standard, a second one is being retooled to prepare it for compliance, and a third is scheduled for evaluation in the near future.

Seal of approval program

The AMHIC Committee on the NAMA Seal of Approval Program has recommended that an approved machine identification program be initiated by NAMA, and the public health and industry groups unanimously concurred with this Committee's recommendation. Furthermore, they recommended that the Seal state "NAMA Approved" unless a legal problem resulted, in which case the wording would refer to "NAMA Listed".

Once the Seal of Approval concept was approved, the AMHIC Committee established the following criteria as to the location of the Seal: (a) one approval statement shall be a part of the cabinet nameplate, (b) a second approval statement shall be affixed to the inside of the cabinet in a readily-visible location, and (c) auxiliary seals may be affixed by the machine manufacturer to other cabinet surfaces as he may elect.

A four-part mailing on this subject has gone out to all machine manufacturers. The mailing included: (a) a revised "Policies" section for administration, (b) a background piece,

(c) a "pro" and "con" report, and (d) a questionnaire soliciting reactions.

A legal research of "NAMA Approved" and preparation of seal artwork are under way at this time, and a full study by the NAMA Board of Directors at its summer meeting will be scheduled since initiation of such a program by the NAMA staff would require Board review.

Carbonation backflow activities

The Secretary of AMHIC reported, in answer to a recommendation by AMHIC in 1971, that he had been unable to find one operating company out of more than 300 queried, that had had carbonation backflow in vented-valve post-mix systems. A representative of a major soft drink beverage industry discussed the testing of non-electric vented valves, which would be needed in directly-connected vending machines such as "slush" venders. He reported the general failure, to date, of such valves.

A public health representative raised the question of why all post-mix vending machines are not equipped with stainless steel precoller tubing instead of copper. The NAMA representative reported that only one company has not made the conversion but pointed out that a copper-free machine without a vented valve, if directly connected to copper tubing outside the machine, would be a retrogression in the performance standards.

In the matter of mandating an incoming water air-gap and reservoir, he further stated that reservoirs in "slush" machines which are not usually serviced by routemen would pose a serious water quality problem.

To help resolve this matter, to better inform the Committee on Carbonation Backflow, and to answer a request of the National Sanitation Foundation who has also been very much concerned with the possibility of poisoning due to carbonation backflow, a summary of NAMA's file of known and suspected copper poisoning cases (from all types of equipment) dating from 1950 has been sent to each member of AMHIC and its respective committees, and to NSF's Backflow Prevention Committee. It is anticipated that a final decision on the type of protection necessary to prevent carbonation backflow in soda fountains and post-mix vending machines will be finalized at the 1973 meeting of AMHIC.

Cut-off controls

At the request of AMHIC, the NAMA Secretary has prepared an excellent proposed booklet entitled, *The Location and Testing of Perishable Food Vending Machine Cut-Off Controls*. This proposed booklet has been submitted to all members of AMHIC and their respective committee for review and comment. Furthermore, this booklet will contain a cautionary statement that the testing methods prescribed herein are intended as field tests rather than definitive or scientific tests which would support regulatory action by a health department. Regardless of this limitation, this booklet should prove a valuable aid to the field sanitarian in determining the workability and the effectiveness of controls to stop the operation of the machine when the air temperature in the food compartment would cause the food to reach a temperature of at least 45 F or more or 140 F or less. This proposed booklet may also be obtained from the National Automatic Merchandising Association.

Icemaker sanitation

The Committee on Icemaker Sanitation has made some extensive studies and deliberated on this matter for several years without arriving at any specific recommendations for incorporating specific requirements in the evaluation manual. This is not intended to be critical of the Icemaker Committee,

but only to emphasize that this is a complex piece of equipment involving several complex problems. Consequently, a background analysis and resume of the AMHIC and industry activities in this matter has been prepared for distribution to committee chairmen. Members of AMHIC recommended that the Committee further explore the relative merits of automatic and manual icemaker cleaning and recommend to AMHIC any action needed. It is anticipated that the Icemaker Sanitation Committee will have prepared specific recommendations for amending the evaluation manual by the 1973 meeting of AMHIC taking into consideration all of the sanitary aspects of ice making and ice vending.

Arthur J. Nolan Public Health Award

The Arthur J. Nolan Public Health Award was created by the NAMA Board of Directors in 1966. The award honors the memory of the late Arthur J. Nolan, an industry leader instrumental in development of the Public Health Service Vending Code and establishment of AMHIC and the vending industry's public health programs. This award is intended to give recognition to those individuals who have made outstanding contributions to the public health programs of the vending industry, and may be presented to any individual who is deemed to be a worthy candidate, other than members of the NAMA staff and retained consultants.

The Arthur J. Nolan Award Committee shall consist of all past Award recipients, with the immediate past recipient serving as Chairman. The functions of the Committee shall be to recommend an annual slate of at least three candidates from which AMHIC would make a final recommendations for the award. These proposed revisions to the Arthur J. Nolan Award have been proposed for addition to the AMHIC's Organization Plan and Procedures

RECOMMENDATIONS

1. The Association reaffirm its support of the National Sanitation Foundation and the National Automatic Merchandising Association and continue to work with these two organizations in developing acceptable standards and education materials for the food industry and public health.

2. The Association urge all sanitarians to obtain a complete set of the National Sanitation Foundation's Food Equipment Standards and Criteria and a copy of the National Automatic Merchandising Association—Automatic Merchandising Health-Industry Council's Vending Machine Evaluation Manual and related materials, to evaluate each of piece of food equipment and vending machine in the field to determine compliance with the applicable sanitation guidelines; and to let this Committee and the appropriate evaluation agency know of any manufacturer, installer, or operator failing to comply with these guidelines.

3. The Association urges all sanitarians and regulatory agencies to support the work of the Association's Committee, submit suggestions for developing new guidelines and for amending same, and subscribe, by law or administrative policy, to the principles represented by the Standards, Criteria, and Evaluation Manual for Food Equipment and Vending Machines.

This report of the Committee on Food Equipment Sanitary Standards respectfully submitted by:

Karl K. Jones, *Chairman*, Purdue University, Student Hospital, West Lafayette, Indiana.

Robert R. Dalton, Michigan Department of Public Health, Lansing, Michigan.

Carl Henderson, State Department of Health and Social Services, Santa Fe, New Mexico.

Howard Hutchings, South Dakota Department of Health, Pierre, South Dakota

O. Donald Moore, Food & Drug Administration, Atlanta, Georgia.

W. Joel Simpson, Pennsylvania Department of Environmental Resources, Tarrisburg, Pennsylvania.

Harold Wainess, Harold Wainess and Associates, Northfield, Illinois.

LETTER TO EXECUTIVE SECRETARY

Mr. H. L. Thomasson
Executive Secretary
I.A.M.F.E.S., INC.
Box 437
Shelbyville, Ind., 46176

Dear "Red":

As the new year approaches and the end of my work rainbow signals my final full-time retirement on January 1, 1974, I look back over the years and realize that my first 25 years in civil government, 23 of these years in the Health Department were some of the most challenging, vigorous and demanding, yet rewarding years of my career.

The late twenties and early thirties brought great changes in the milk industry through new laws, milk strikes, depression and what have you, and particularly milk sanitation. Although my work entailed direction and supervision of the food, meat, milk and general sanitation programs of the city, milk sanitation was granted the highest priority with the three health officers under whom I served. In 1950 I tired of the increasing directions from City Hall to do virtually the impossible with limited staff and low wages and the inability to educate my children under these conditions.

I left the Department on the advice of my physician who understood the demanding and thankless position I was in who gave me these historic words, "If you stay on this job, I can't guarantee that you won't be six feet under in six months." In taking his advice I had to forego my pension four years away. This was really the best thing that ever really happened to me as I had no pension to fall back on and "rot away" as the doctor said. The change of pace restored my health allowing me to continue in various phases of the milk industry for the next 23 years now terminating as executive secretary of the Rochester Milk Dealers Association.

I want to take this opportunity to congratulate you on the outstanding success of the N.Y.A.M.F.S. and I.A.M.F.E.S. celebrating jointly their 50th and 60th anniversaries respectively in August in Rochester, N. Y. These two events just happened to coincide with my 74th anniversary on August 12th. My part on the local committee was merely incidental. As postmortems, I heard many compliments on the programs and the manner in which they were conducted. I am sure you played an important part in master-minding these affairs. As I heard of your announcement to retire and turn over the reins soon, I said to myself, "with 'Red' goes the passing of a glorious era in the field of sanitation." May your retirement years be filled with Fair Weather, Blue Skies and Good Sailing in the future ahead.

Sincerely, George West, Executive Secretary

THIRD EDITION OF HARVESTING YOUR MILK CROP

The third edition of *Harvesting Your Milk Crop*, by eminent dairy scientist Dr. Charles W. Turner is now available to the dairy concerned public.

Dr. Turner clearly illustrates and discusses how udder formation along with its proper preparation influences milk secretion. With the changeover from hand milking to machine milking, he also explains how the construction and operation of milking machines affects the milk harvest. At a time when milk prices are at all time highs, the value of a pound of milk is greater than ever. The information in this book, when properly implemented, can mean many more pounds of milk per cow per year.

Published by Babson Bros. Dairy Research Service, this new edition contains additional information covering the latest development in automated milking systems—including the effects on cow milking by automated prep stalls and automatic milkers.

The first edition, published in 1962, was read by thousands of dairymen and others concerned with the production of dairy products. It was also translated into Spanish and made available to Latin Ameri-

can dairymen by the Rockefeller Foundation.

Dr. Turner is the author of numerous scientific papers, bulletins and textbooks. Currently he is professor emeritus in dairy husbandry at the University of Missouri, Columbia.

The latest edition may be obtained from Surge Dairy Equipment dealers, everywhere, or by sending a letter directly to Babson Bros. Dairy Research Service, 2100 S. York Road, Oak Brook, Illinois 60521. The cost of the book is \$1.00 in the United States and \$1.50 in other countries.

BAKERY EXPO '73 OUTSTANDING SUCCESS

"Bakery Expo '73 was an outstanding success," states E. Archer Turner, Exposition Chairman. "This was the largest national and international trade show ever presented by and for the baking industry," Chairman Turner reported.

There were more exhibitors, more space used, and the largest attendance than ever before. The latest tally discloses, that in addition to every state in the Union, 48 foreign countries were represented. A detailed audit is being prepared for Committee consideration and will be reported on in the near future.

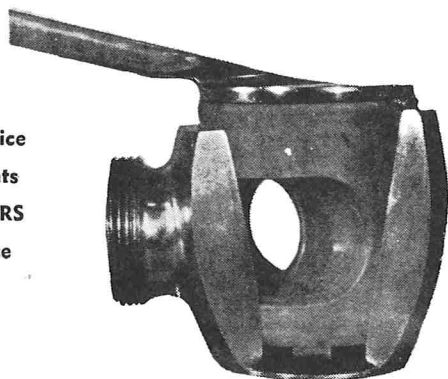
The new BEMA Bakery Equipment Guide was initially distributed at the Exposition and is being widely disseminated. This "Guide" presents prominent bakery equipment and machinery manufacturers who compose the membership of the Bakery Equipment Manufacturers Association. The first section lists the member companies and the full line of products each manufactures for the baking industry. The second section presents these products in alphabetical order, listing the manufacturers thereof in each category.

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COMPILED AND EDITED BY

J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH

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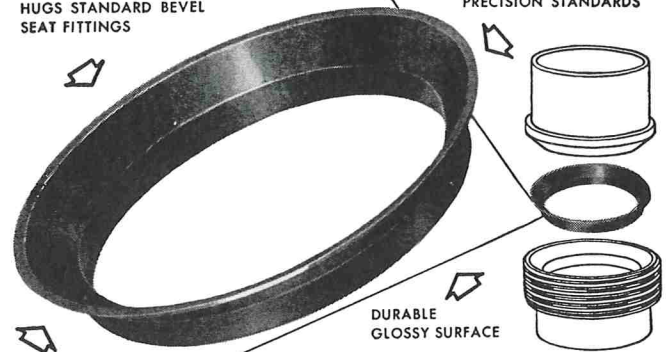
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Dairy authorities speak out on better cow milking



Dr. Elmer H. Marth
Department of Food Science,
University of Wisconsin/Madison

Rapid cooling can help maintain high quality of raw milk

The two most important things a dairyman can do to insure milk quality at his dairy are:

1. Put the cleanest possible milk into the bulk tank.
2. Cool it as quickly and efficiently as possible.

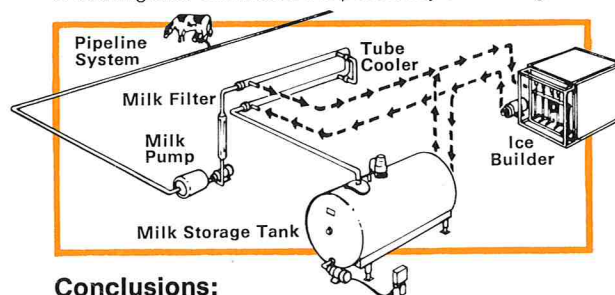
Much has been said about the necessity of sanitary milk handling through the entire milking operation. However, even under the most sanitary conditions, milk from a healthy cow will contain several hundred to several thousand bacteria per milliliter. Certain strains can cause undesirable conditions such as rancidity or other off-flavors unless their growth is retarded.

Follow the rules

The best way to retard bacterial growth is by cooling milk as rapidly as possible, without freezing it.

1. *Milk must be cooled promptly.* Delays result in bacterial growth. Some of the bacteria in milk can multiply in as little as 20 to 30 minutes if the milk is warm.
2. *Cooling should be rapid,* so further appreciable bacterial growth does not occur during the cooling process. Care must always be exercised so that milk does not freeze.
3. *Milk must be cooled to and maintained at a safe temperature.* Cooled milk must be held at a 40° F. and preferably 36-38° F. This temperature must be maintained throughout the storage period. When freshly drawn milk is added to milk already in the bulk tank, the rise in temperature of the initial milk must be minimal and the temperature of all milk in the tank must be rapidly reduced to 36-38° F. (Again, milk must not be frozen in the process.)
4. *Raw milk should not be stored for excessive periods* and should be moved from the bulk tank to the tank truck under conditions which preclude additional microbial contamination.

The refrigerated bulk cooling tank is the most widely used device to cool milk on the farm today. However, it is limited in its ability to meet some of the demands outlined above. New equipment available makes it possible to "pre-cool" milk on the way to the tank. Instant coolers using chilled water from an ice-builder help make rapid cooling practical on the farm. This type of cooling also eliminates the possibility of freezing milk.



Conclusions:

Even though all conditions needed for effective rapid cooling are met, some bacteria can still grow in refrigerated milk. Two points already discussed bear repeating. Be certain that good sanitary practices are followed during production of milk to insure that few bacteria of the kind able to grow at refrigeration temperatures are present. Do not hold raw milk refrigerated for excessive periods.

The successful marketing of milk depends on everyone doing his part at each step along the way. Even though you, as an individual dairyman, may not benefit *directly* from each of your efforts to improve sanitation and milk handling, you most certainly will benefit *indirectly* through greater total consumer acceptance of milk and dairy products thanks to fewer flavor problems and the absence of other negative factors caused by improper handling.

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