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Journal of Milk and Food Technology

61ST ANNUAL MEETING

ST. PETERSBURG HILTON

August 12, 13, 14, 1974

St. Petersburg, Florida

(Reservations Page 1)

Official **Publication**

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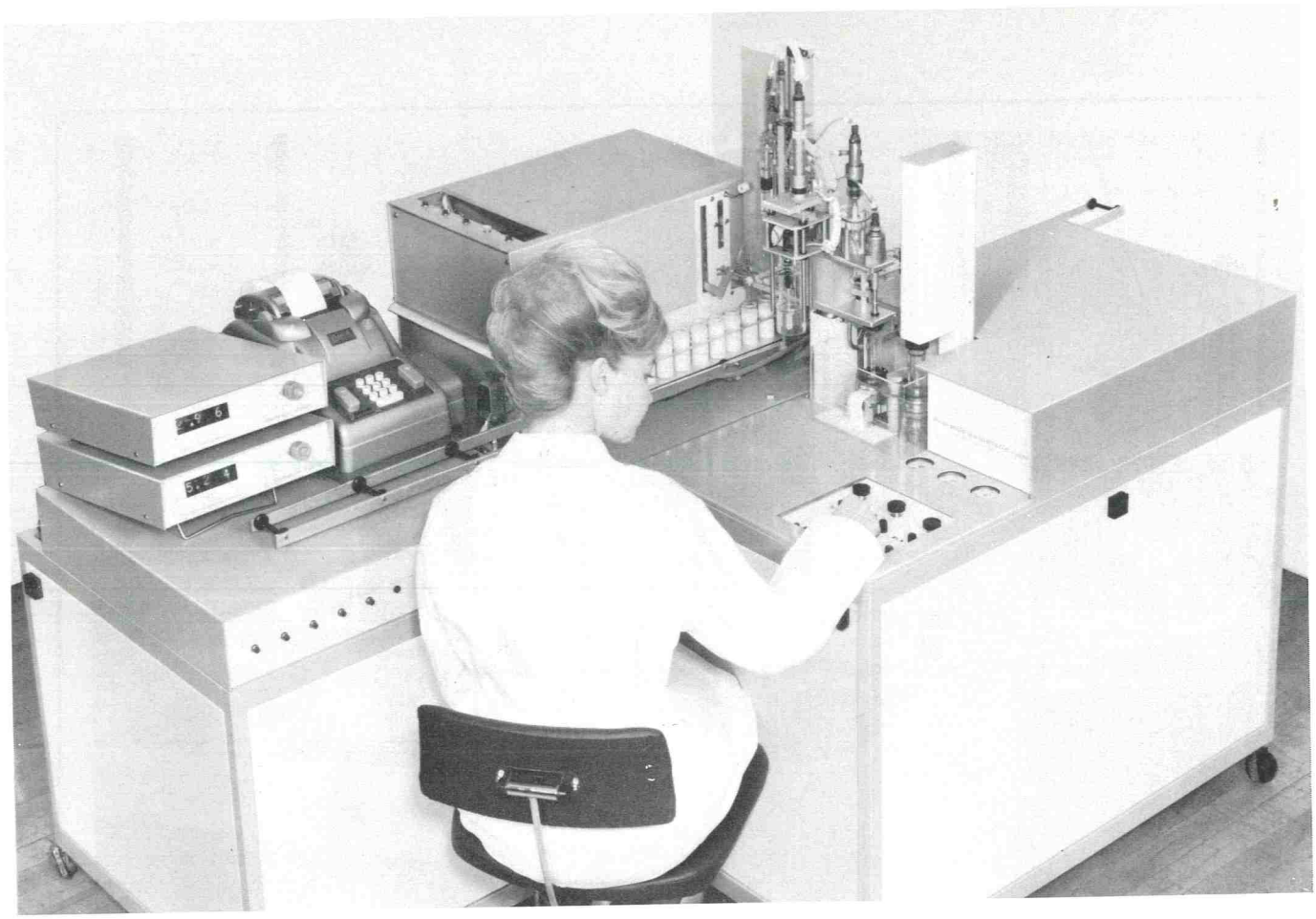

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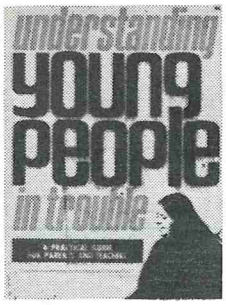

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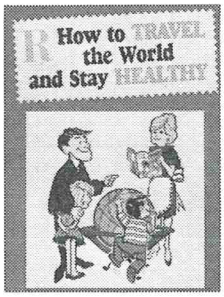

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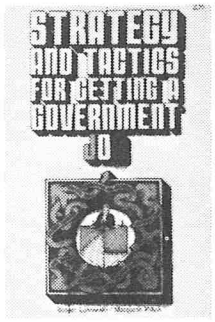

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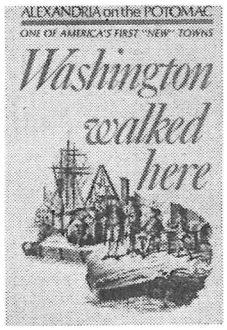

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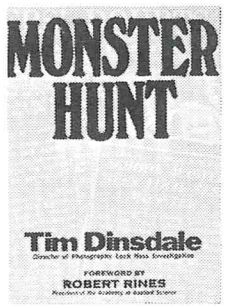

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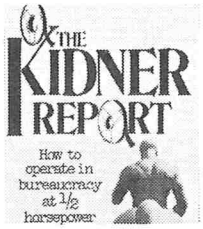

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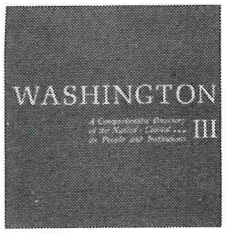

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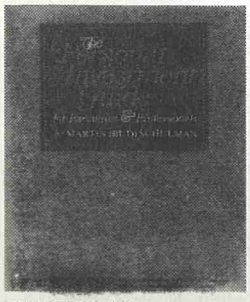

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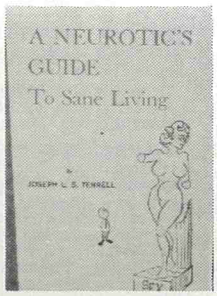

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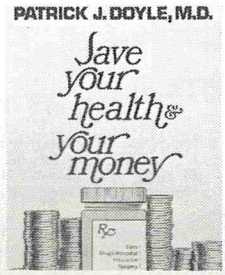

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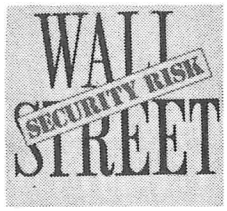

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THE ROLE OF *BACILLUS CEREUS* IN SWEET CURDLING OF FLUID MILK

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Tennessee Agricultural Experiment Station, Knoxville 37901

(Received for publication December 26, 1973)

Abstract

Twenty-eight percent of commercially pasteurized milk samples procured from various plants throughout Tennessee exhibited sweet-curdling within 10 days on refrigerated storage. Psychrotrophic *Bacillus cereus* was isolated as a causative organism from these milk samples. The isolates differed from the type culture organism only in their ability to grow and bring about sweet curd formation in skimmilk at refrigeration temperature. These isolates exhibited marked differences in their response to heat activation temperatures as well as to the initial excessive growth of *Pseudomonas* species in raw milk. Spores of three isolates exhibited greater activity after activation at 80 C for 15 sec than at the standard pasteurization temperature of 71.5 C for 15 sec. Excessive growth of *Pseudomonas fragi* or *Pseudomonas fluorescens* in raw skimmilk before processing had a stimulatory effect on two of three psychrotrophic *Bacillus cereus* isolates in combination with heat-activation especially with activation at 80 C for 15 sec.

Recent developments in processing and storing milk have emphasized the importance of sporeforming organisms in the raw milk supply. Although psychrotrophic *Bacillus* species were recovered from soil and water *(8)*, it was not until 1969 that Grosskopf and Harper *(5)* reported the isolation of psychrotrophic sporeforming bacteria from pasteurized milk. Following this report, many isolations have been made from milk *(3, 4, 11, 12)* and the percent of raw milk samples containing psychrotrophic sporeformers has been reported as ranging from 25 to 84 *(4, 5, 12)*.

Defects caused by sporeformers have commonly been associated with highly heated milk and milk products, and the most prevalent among these defects has been the so-called "sweet curd." More recently, this defect has occurred in pasteurized fluid milk that has been stored for several days at refrigeration temperatures. The first manifestation of this defect in pasteurized milk is the formation of a curd on the bottom of the carton that, in many instances, goes undetected by the consumer.

Hammer and Babel *(6)* suggested that the sweet-curdling defect was caused by a rennin-like enzyme produced by sporeforming bacteria. Jayne-Williams and Franklin *(7)* found *Bacillus cereus* to be related to this defect. Martin et al. *(9)* reported *B. cereus* represented 37% of the 10 *Bacillus* species isolated from raw milk.

This study was undertaken to determine the influence of the trend in the dairy industry toward higher pasteurization temperatures and extended storage times on activation, germination, and outgrowth of psychrotrophic *B. cereus,* and to determine any stimulatory influence of the growth of the common *Pseudomonas* organisms in raw milk on these spores.

Materials and Methods

Media

Mannitol-Egg Yolk-Phenol Red-Polymyxin Agar (MYP) *(10)* was used to isolate psychrotrophic *B. cereus.* Nutrient Agar containing 1.0% soluble starch and 0.01% manganese sulfate was used as a sporulation medium. Standard Methods (SM) Agar containing 0.1% soluble starch was used for making counts of *B. cereus.* Trypticase Soy (TS) Agar and Trypticase Soy (TS) broth were used to isolate *Pseudomonas fluorescens* and *Pseudomonas fragi.*

Bacterial cultures

Ten isolates of *B. cereus* were obtained from pasteurized fluid milk that had been refrigerated 8 to 10 days and had developed sweet-curdling. Plates of MYP agar were spread with 0.1 ml of the sweet-curdled milk of the appropriate dilutions and incubated at 35 C for 24 to 48 h. Rough, dry, discrete colonies with a distinct, violet-red background, surrounded by a halo of dense white precipitate were picked into TS broth.

These isolates were purified and conformed to the characteristics of *B. cereus (2)* and were identical to *B. cereus* ATCC 14579 except they grew and produced sweet-curd in milk within 10 days at 5 or 10 C *(1)*.

The *P. fluorescens* and *P. fragi* organisms were isolated from pasteurized milk using TS agar, identified *(2)*, and after purification served as the isolates.

Spore preparation

Stock cultures of *B. cereus* were maintained by semi-monthly transfers on TS agar slants. Transfers from the stock cultures were made into TS broth and incubated for 18 h at 35 C. Nutrient agar (125 ml) was used for slants in Roux flasks, inoculated with 2 ml of the stock culture and incubated at 35 C for 7 days. The spores were washed from the slants with 0.01 M phosphate buffer (pH 7.2). After four washings with the buffer, the spores were incubated for 48 h at 10 C with papain extract (0.4 mg/ml) in 0.05 M phosphate buffer (pH 7.0) containing sodium thioglycolate (0.5 mg/ml) and 1:1 toluene and chloroform mixture (0.5%). Following the papain treatment and three washings with glass distilled water the spores were suspended in 0.01 M phosphate buffer and stored at refrigerated temperature until needed.

[1]Present address: University of Agricutural Sciences, Hebbal, Bangalore 560024, India.

Heat-activation of B. cereus spores

Turbidimetrically standardized spore preparations were added to sterile skimmilk at the 1% level and heat-activated as follows: *(a)* 80 C for 15 sec, *(b)* 85 C for 10 min, or *(c)* 71.5 C for 15 sec. All preparations were immediately cooled to room temperature in ice water.

Inoculation of various sterile skimmilks

The following sterile skimmilks *(a)* plain skimmilk (autoclaved at 121 C for 6 min), *(b)* skimmilk in which P. *fluorescens* was grown and subsequently autoclaved as above, and *(c)* skimmilk in which *P. fragi* was grown and subsequently autoclaved as above, were inoculated with 0.6 to 1% of the heat-activated spore suspensions. Duplicate 1-ml quantities of the autoclaved milk were plate on Standard Methods (SM) agar and incubated for 48 h to determine sterility.

Plate counts of the skimmilks inoculated with heat-activated spores

After inoculation with spores, skimmilks were incubated at 35 C for up to 11 h. Plate counts were made in duplicate on SM agar with 0.1% soluble starch beginning with the initial count (0 h) and after 4, 6, 7, 8, 9, 10 and 11 h of incubation.

RESULTS AND DISCUSSION

Fifty-four samples of commercially pasteurized milk were obtained from various plants throughout Tennessee over a period of a year, and 28% developed the sweet-curdling defect on refrigerated storage (5 to 7 C) within 8 to 10 days. In many of these cartons of milk curd was present only at the bottom, but on occasion, the entire milk was curdled. The pH of the sweet-curdled samples was between 6.2 and 6.6. Because of this curdling on the bottom of the cartons, a series of preliminary trial with *B. cereus* ATCC 14579 was conducted to determine the distribution of the organisms in various portions of skimmilk. Sterile skimmilk in sterile, tall glass cylinders was inoculated with heat activated spores to obtain an initial count of 650,000/ml and incubated at 10 C. Results of the plate counts of four replicates taken on 3, 6, and 9 days from the top, middle, and bottom portions are presented in Table 1. The higher counts were from the bottom positions in the samples indicating that the defect first appeared at the bottom of cartons of milk.

Of 10 isolates of *B. cereus* from milk that exhibited sweet-curdling in 10 days at refrigeration temperatures, CH-1, BC-5, and H-3 were selected for study. The cultural, morphological, and biochemical properties of the isolates were identical to those of the type culture *B. cereus* 14579 except that the type culture failed to produce sweet curdling at 5-10 C even after 20 days of incubation.

Trials with isolate CH-1

Sterile skimmilk was inoculated with 3,000 to 6,700 activated spores of CH-1/ml. The spores in six replicates were added after activation at the three temperature-time combinations and after the growth of *P. fluorescens* or *P. fragi*. Results from these trials are shown in Tables 2, 3, and 4. Even though data in these tables suggest a higher plate count with spores activated at 80 C for 15 sec in the sterile skimmilk, the counts from the three different methods of spore activation were not significantly different. However, with spores activated at 80 C for 15 sec, counts were significantly different at the 5% level in the milk in which *P. fluorescens* or *P. fragi* was grown before sterilization. The Duncan's new multiple-range test was used to compare each treatment mean with every other treatment mean and the analyses are presented in Table 5. This statistical evidence shows that the previous growth of *P. fragi* or *P. fluorescens* in skimmilk had a greater stimulatory effect on the CH-1 spores when activated at 80 C for 15 sec than had the previous growth of *P. fluorescens* and activation at 71.5 C for 15 sec.

Trials with isolate BC-5

Results from six replicates with isolate BC-5 and

TABLE 1. AVERAGE PLATE COUNTS OF *Bacillus cereus* ATCC 14579[a] AT THE TOP, MIDDLE AND BOTTOM PORTIONS OF MILK AT DIFFERENT INTERVALS OF INCUBATION

Location of the aliquot in the cylinder	3 days	6 days	9 days
	(in millions)[b]		
Top	1.6	1.4	1.36
Middle	1.6	3.4	10.00
Bottom	3.8	8.0	15.00

[a]Activated at 85 C for 10 min.
[b]Average of four replications.

TABLE 2. AVERAGE PLATE COUNTS OF ISOLATE CH-1 ACTIVATED AT 80 C FOR 15 SEC AT VARIOUS INTERVALS OF INCUBATION IN STERILE MILK AND IN MILKS IN WHICH *Pseudomonas* SPECIES GREW PRIOR TO STERILIZATION

Hours of incubation	Plate counts[a]		
	CH-1	FrCH-1	FlCH-1
	(In millions)[b]		
4	1.34	1.22	1.29
6	11.31	12.38	12.26
7	18.86	20.58	20.38
8	28.18	30.05	27.50
9	36.05	38.66	36.51
10	49.00	51.13	49.70
11	59.50	62.08	64.83

[a]CH-1 = Plain sterile skimmilk in which activated spores of CH-1 were inoculated. FrCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of CH-1. FlCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of CH-1.
[b]Average of six replications.

TABLE 3. AVERAGE PLATE COUNTS OF ISOLATE CH-1 ACTIVATED AT 85 C FOR 10 MIN AT VARIOUS INTERVALS OF INCUBATION IN STERILE MILK AND IN MILKS IN WHICH *Pseudomonas* SPECIES GREW PRIOR TO STERILIZATION

Hours of incubation	Plate counts[a]		
	CH-1	FrCH-1	FlCH-1
	(In millions)[b]		
4	1.70	1.75	1.61
6	9.69	10.35	9.70
7	14.41	13.05	13.96
8	22.17	22.10	22.71
9	26.80	31.42	30.56
10	36.50	38.88	36.63
11	45.10	50.88	48.28

[a]CH-1 = Plain sterile skimmilk in which activated spores of CH-1 were inoculated. FrCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of CH-1. FlCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of CH-1.

[b]Average of six replications.

TABLE 4. AVERAGE PLATE COUNTS OF ISOLATE CH-1 ACTIVATED AT 71.5 C FOR 15 SEC AT VARIOUS INTERVALS OF INCUBATION IN STERILE MILK AND IN MILKS IN WHICH *Pseudomonas* SPECIES GREW PRIOR TO STERILIZATION

Hours of incubation	Plate counts[a]		
	CH-1	FrCH-1	FlCH-1
	(In millions)[b]		
4	2.02	0.80	0.70
6	11.05	8.15	7.63
7	17.30	12.60	11.25
8	26.96	19.20	17.10
9	29.51	26.15	22.02
10	39.73	34.70	28.68
11	48.60	44.08	37.96

[a]CH-1 = Plain sterile skimmilk in which activated spores of CH-1 were inoculated. FrCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of CH-1. FlCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of CH-1.

[b]Average of six replications.

initial inocula of 3,000 to 4,300/ml in similar trials are shown in Tables 6, 7, and 8. Here again, as with CH-1, there was a strong indication of greater activity of the spores after activation at 80 C for 15 sec but the differences were not statistically significant. The result of analysis of variance for heat activation and influence of initial growth of *Pseudomonas* species on germination and outgrowth of BC-5 was significant at the 1% level. Duncan's new multiple range test comparing each treatment mean with every other treatment mean is presented in Table 9. The stimulatory effect of *P. fluorescens* is strikingly evident with BC-5 when activated at 80 C for 15 sec and this treatment was different from all other treatments at the 1% level.

Trials with isolate H-3

The average plate counts of the H-3 isolate activated at the three different treatments, when the initial spore counts varied from 3,300 to 4,700/ml, were not statistically different from each other. Although there were differences in the counts as a result of the treatments used, these counts were not significant even at the 5% level.

TABLE 5. TREATMENTS AND THE TREATMENT MEANS OF ISOLATE CH-1

Treatment	Treatment mean
FrCH-1 and activation at 71.5 C for 15 sec	18.2219[ab]
FlCH-1 and activation at 71.5 C for 15 sec	15.6722[a]
CH-1 activated at 71.5 C for 15 sec	21.9202[ab]
FrCH-1 and activation at 85 C for 10 min	21.0588[ab]
FlCH-1 and activation at 85 C for 10 min	20.4346[ab]
CH-1 activated at 85 C for 10 min	19.5686[ab]
FrCH-1 and activation at 80 C for 15 sec	27.0152[b]
FlCH-1 and activation at 80 C for 15 sec	26.5623[b]
CH-1 activated at 80 C for 15 sec	25.5328[b]

[a, ab, b]Numbers having the same superscript are not significantly different ($P < 0.5$) by Duncan's multiple range test. FrCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of CH-1. FlCH-1 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of CH-1. CH-1 = Plain sterile skimmilk in which activated spores of CH-1 were inoculated.

TABLE 6. AVERAGE PLATE COUNTS OF ISOLATE BC-5 ACTIVATED AT 80 C FOR 15 SEC AT VARIOUS INTERVALS OF INCUBATION IN STERILE MILK AND IN MILKS IN WHICH *Pseudomonas* SPECIES GREW PRIOR TO STERILIZATION

Hours of incubation	Plate counts[a]		
	BC-5	FrBC-5	FlBC-5
	(in millions)[b]		
4	1.35	1.02	1.73
6	12.71	13.00	23.58
7	20.35	21.75	46.16
8	27.40	27.25	62.66
9	38.41	36.56	77.66
10	46.08	43.70	98.41
11	61.88	65.50	98.41

[a]BC-5 = Plain sterile skimmilk in which activated spores of BC-5 were inoculated. FrBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of BC-5. FlBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of BC-5.

[b]Average of six replications.

TABLE 7. AVERAGE PLACE COUNTS OF ISOLATE BC-5 ACTIVATED AT 85 C FOR 10 MIN AT VARIOUS INTERVALS OF INCUBATION IN STERILE MILK AND IN MILKS IN WHICH *Pseudomonas* SPECIES GREW PRIOR TO STERILIZATION

Hours of incubation	Average plate counts[a]		
	BC-5	FrBC-5	FlBC-5
	(in millions)[b]		
4	1.02	1.00	0.88
6	9.47	9.28	9.78
7	17.35	16.60	17.63
8	25.53	23.97	29.33
9	35.01	34.81	38.48
10	44.45	45.05	45.53
11	56.00	56.41	58.68

[a]BC-5 = Plain sterile skimmilk in which activated spores of BC-5 were inoculated. FrBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of BC-5. FlBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of BC-5.
[b]Average of six replications.

TABLE 8. AVERAGE PLACE COUNTS OF ISOLATE BC-5 ACTIVATED AT 71.5 C FOR 15 SEC AT VARIOUS INTERVALS OF INCUBATION IN STERILE MILK AND IN MILKS IN WHICH *Pseudomonas* SPECIES GREW PRIOR TO STERILIZATION

Hours of incubation	Average plate counts[a]		
	BC-5	FrBC-5	FlBC-5
	(in millions)[b]		
4	1.56	1.34	1.41
6	10.00	8.16	8.53
7	18.91	13.88	18.01
8	24.88	19.61	29.03
9	33.40	27.70	39.13
10	40.26	37.75	45.36
11	49.45	51.55	55.33

[a]BC-5 = Plain sterile skimmilk in which activated spores of BC-5 were inoculated. FrBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of BC-5. FlBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of BC-5.
[b]Average of six replications.

Variations that can be seen in the results from these three isolates may be attributed to strain difference and activation temperature requirements. Since pasteurization temperatures have been raised above the required limits in many plants, these results help explain why commercially pasteurized milk shows the sweet-curd defect on refrigerated storage.

In conclusion, these results provide evidence that contributes to the knowledge of sweet-curdling in fluid milk. First, the psychrotrophic sporeformer, *B. cereus* was a causative organism. Second, higher temperatures currently used in the pasteurization of milk were more effective in activating the spores of this organism than the standard pasteurization temperature. Finally, the previous growth of psychrotrophic *Pseudomonas* species coupled with the higher pasteurization temperatures may stimulate germination and outgrowth of certain *B. cereus* strains.

TABLE 9. TREATMENTS AND THE TREATMENT MEANS OF ISOLATE BC-5

Treaments	Treatment mean
FrBC-5 and activation at 71.5 C for 15 sec	20.0014[a]
FlBC-5 and activation at 71.5 C for 15 sec	24.6047[a]
BC-5, activation at 71.5 C for 15 sec	22.3108[a]
FrBC-5 and activation at 85 C for 10 min	23.3926[a]
FlBC-5 and activation at 85 C for 10 min	25.0421[a]
BC-5, activation at 85 C for 10 min	23.5224[a]
FrBC-5 and activation at 80 C for 15 sec	26.0990[a]
FlBC-5 and activation at 80 C for 15 sec	49.8107[b]
BC-5, activation at 80 C for 15 sec	26.0251[a]

[a,b]Numbers having the same superscript are not significantly different by Duncan's Multiple Range Test at 1% level.
FrBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fragi* and subsequently inoculated with activated spores of BC-5.
FlBC-5 = Skimmilk which was sterilized after growth of *Pseudomonas fluorescens* and subsequently inoculated with activated spores of BC-5.
BC-5 = Plain sterile skimmilk in which activated spores of BC-5 were inoculated.

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A HOT ACID TREATMENT FOR ELIMINATING *SALMONELLA* FROM CHICKEN MEAT

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Abstract

Hot succinic acid was tested for destruction of *Salmonella* on chicken broilers. Immersion in a 3% succinic acid solution at 60 C resulted in some destruction of *Salmonella montevideo* (200 cells inoculated per leg), but an acid treatment at 85 C for 2-3 min was required to achieve elimination. However, when 200 cells of S. *montevideo* were inoculated into either a chicken meat or skin homogenate a temperature of only 60 C for 1 and 2 min, respectively, was required for elimination.

A number of attempts have been made employing moist heat to destroy salmonellae on poultry carcasses. Klose et al. *(9)*, employing subatmospheric steam at 66 C for 8 min or 71 C for 4 min, reported substantial reductions in numbers of *Salmonella typhimurium* that had been spray inoculated on whole broiler carcasses, but only limited reduction on carcasses that had been inoculated by soaking. Avens and Miller *(1)* reported that the heat resistant *Salmonella senftenberg* 775 W was eliminated by immersion of excised turkey skin samples in lactic acid (5%) at 72 C or 60 C for 5 sec and at 28 C for 15 sec, but could not be eliminated from turkey drumsticks, half carcasses, or whole carcasses with these treatments. The effect of these treatments on salmonellae other than S. *senftenberg* 775 was not reported.

A recent investigation in this laboratory [Cox et al. *(6)*] demonstrated a significant reduction in total bacterial count and an extension of shelf life of broiler legs after immersion in succinic acid (3%) at 60 C for 3 min. The objective of the study reported herein was to evaluate the use of a hot succinic acid dip for eliminating *Salmonella* from broiler legs.

Materials and Methods

Destruction of cells on a chicken part

Salmonella montevideo cells were grown on brain-heart infusion agar (Difco) slants for 18 h and then washed from the culture medium with sterile phosphate-buffered diluent *(5)*. The suspension was diluted with additional phosphate diluent until optical density (OD) was 0.2 at 540 nm with a Baush and Lomb Spectronic 20 Spectrophotometer.

Legs (thigh and drumstick) were aseptically removed from freshly processed broiler carcasses and placed on aluminium foil with the inner surface of the thigh facing downward. This piece was obtained by a cut through the junction of the thigh muscles with the pelvic girdle to the hip joints disjointing the femur. The loin or "oyster" muscle was left on the back.

Each leg was inoculated with a suspension of S. *montevideo*, that had been serially diluted to give ca. 2,000 cells/-ml (enumerated by spreading 0.1-ml aliquots on plates of brain-heart infusion agar). One-tenth milliliter of the inoculum was spread thoroughly by rubbing for 1-2 min with a sterile bent glass rod, over a skin area of about 30 cm^2 (near the thigh and drumstick junction), from which excess moisture was previously removed with sterile tissue paper. This concentration of cells (200) was selected because of reports by Surkiewicz et al. *(10)* and Idziak and Incze *(8)* that most naturally contaminated carcasses contain low levels of salmonellae.

The inoculated legs were immersed, four at a time, into a 3% succinic acid solution at selected temperatures between 60 and 85 C for 1, 2, or 3 min. They were then immediately dipped into 24 C tap water for 1 min. For control samples, water of the same temperatures was used instead of succinic acid in the first dip. Different containers and fresh solutions were used for each treatment.

Salmonella determinations were done as follows: Individual legs were put in 27-oz sterile Whirl-Pak bags and 100 ml of lactose broth, containing 0.6% (v/v) tergitol anionic 7 were added. The contents were shaken for 1 min and four 10-ml aliquots of this lactose broth rinse were removed and incubated for 24 h at 35 C. The remainder of the lactose broth rinse containing the sampled leg was also incubated for 24 h at 35 C. After incubation, the four 10-ml aliquots and 10 ml from the incubated leg sample were each added to 100 ml of TT broth of Hajna and Damon *(7)*.

Methods, media and incubation procedures used for identification of *Salmonella* appear in Table 1. Cultures showing a typical biochemical pattern and giving agglutination with Salmonella H antisera poly a - z, s and m as well as with Salmonella O antisera poly A - I and group C_1 - factor 7 were taken to be S. *montevideo*. By this procedure, the effect of the various treatments on the artificially inoculated cells could be studied apart from the "naturally" present salmonellae. A leg was considered positive for *Salmonella* if any of the five aliquots were positive for S. *montevideo*. All media were prepared from complete dehydrated products (Difco).

De truction of cells in homogenates

Ground chicken meat was mixed with an equal weight of sterile 0.3 M KH_2PO_4 aqueous solution at 5-10 C, then the mixture was blended at high speed and filtered through a sterile metal sieve (18 mesh) to produce a homogenous

TABLE 1. MICROBIOLOGICAL METHODS USED FOR DETECTING *Salmonella*

Medium	Incubation time (h)	Incubation temp. (C)	Method
Lactose broth (Difco) with 0.6% (v/v) Tergitol Anionic 7[a]	24	35	Pre-enrichment
TT broth (Difco)[b]	24	35	Enrichment
Brilliant green agar (Difco) with 80 mg/l sodium sulfadiazine	24	35	Isolation
Triple sugar iron agar, Lysine iron agar, urea broth (Difco)	24	35	Biochemical confirmation
O and H antisera (Difco)	24	35	Serological confirmation

[a]Union Carbide Corp., Chicago, Ill.

[b]Tetrathionate Broth Base Hajna

slurry. Chicken skin homogenates were prepared in the same way. Bacteriological analysis of the homogenates showed that they contained no *Salmonella*.

S. montevideo cells were grown on brain heart infusion agar slants for 18 h and then washed from the culture medium with sterile phosphate-buffered diluent. This suspension was mixed thoroughly with the homogenate to give a final concentration of 4×10^3 cells of *S. montevideo* per milliliter. The final pH of the mixture was 6.5.

Capillary tubes of 0.08 ml capacity (150 mm long and 0.83 mm internal diameter) were filled aseptically with 0.05 ml of the meat or skin slurry containing 200 cells of *S. montevideo*. The capillary tubes were cleaned before use by soaking for 24 h in a mixture of ethyl alcohol and 3 N HCl (1:1), rinsed thoroughly with distilled water then with acetone, and then dried at 50 C overnight.

Tubes were filled by immersing one end in the homogenate and then applying suction at the other end with a hypodermic syringe. The tubes were then sealed at both ends with a gas flame, care being taken not to heat the homogenate. Tubes were then wiped, dipped for 5 min in a 0.3% chloramine-T (n-sodium-n-chloro-p-toluene-sulfonamide) solutions, rinsed with sterile tap water, and dried with sterile tissue paper.

Multiplication of the test organism during the preparation time was prevented by keeping the temperature of the inoculated homogenate below 5 C.

Eight capillaries containing the meat or skin homogenates were heated simultaneously in a water bath at 50, 55, 60, 65, or 70 C for 1, 2, or 3 min, and then rapidly cooled in ice water. Less than 10 sec were required to equilibrate the temperature of the capillary contents (as determined by thermocouples) with that of the heating/cooling bath. The tubes were dried with sterile tissue paper and their contents transferred to test tubes containing 10 ml of lactose broth, 0.6% tergitol anionic 7 added. The ends of each capillary were broken with a flamed forceps and the contents forced out one end by filtered compressed air applied with a hypodermic needle. The air was filtered through a sterile MHWGO3700 monitor having a 0.45 μ pore size membrane filter (Millipore Corporation, Bedford, Mass.). The capillaries were also added to the broth. To prevent cross-contamination, the needle was immersed between samples in a 0.3% Chloramine-T solution for 2-3 min and then rinsed with sterile tap water. The lactose broth tubes were incubated for 48 h at 35 C. Procedures for identification of *S. montevideo* were the same as those previously described.

RESULTS AND DISCUSSION

Chicken parts

Inoculated *S. montevideo* was recovered from 12 out of 12 legs after a 3-min dip in either water or 3% succinic acid solution at 60 C. However, when an analysis was made of the 48 aliquots per treatment (four 10-ml aliquots per leg), the number of *S. montevideo*-positives were 44 for the water and 11 for the acid. These results show that when degree of destruction rather than elimination is considered, succinic acid was more effective again *Salmonella* than water in a 3-min immersion at 60 C.

The possibility of eliminating *Salmonella* from the raw chicken legs using a 3% succinic acid solution at temperatures higher than 60 C was investigated. Results of this are shown in Fig. 1. Immersion in the acid solution at 85 C for 2 min was required for complete elimination of artificially inoculated *S. montevideo* from fresh chicken legs (a). After im-

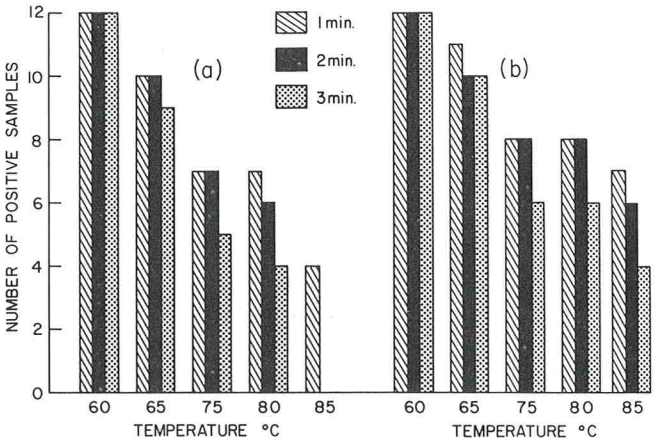

Figure 1. Recovery of artificially inoculated *S. montevideo* from 12 chicken legs after immersion in a (a) 3% succinic acid or (b) water.

mersion in water at 85 C for 2 or 3 min, S. *montevideo* was recovered from 50 and 35% of the samples, respectively (b).

The skin of all 60 C-treated parts exhibited a partially cooked appearance, whereas those subjected to 85 C had a definite cooked appearance.

The above results would appear to be inconsistent with data from other studies [Beloian and Schlosser *(3)* and Bryan et al. *(4)*] which indicate that salmonellae, except for S. *senftenberg* 775 W, can be rather easily destroyed in foods by exposure to temperatures of about 60-65 C for a few minutes. This apparent conflict is even more striking when one considers that even the very low cell inoculum could not be eliminated by exposure to the succinic acid for 3 min at 80 C. To determine whether the particular S. *montevideo* used in our study possessed exceptional heat resistance in ground chicken meat or if chicken skin per se had exerted a protective effect on the *Salmonella*, destruction of our test organism in ground chicken meat and in skin homogenates was studied.

S. *montevideo* was recovered from all samples of skin or meat homogenates heated at 50 and 55 C for 1, 2, or 3 min. None of the organisms were recovered from the meat homogenates heated at 60 C or higher, regardless of time of exposure. In the skin homogenates, however, the test organism was recovered from 2 of 8 samples heated at 60 C for 1 min, but not from samples heated for 2 or 3 min at this temperature, nor from any of those heated at 65 or 70 C.

These results are in essential agreement with those reported by Bayne et al. *(2)*, who observed destruction of 3×10^8 cells of S. *typhimurium* in ground pectoral muscle after 5 min at 60 C and indicates that our test organism did not possess exceptional heat resistance. Our data also indicate that chicken skin, per se, does not alter the heat resistance of *Salmonella* and as such, support the inferences drawn by others *(1, 9)* that adequate exposure of salmonellae to heat or a bactericidal fluid may be the limiting factor in effectively pasteurizing whole carcasses or parts. In addition to inefficient conduction of heat and "pockets" of salmonellae contamination that may exist at certain locations in whole carcasses, e.g. the visceral cavity *(9)*, our data suggest that factors associated with the location of these organisms on the skin in situ mitigate the effect of normal pasteurization procedures.

The effective acid-heat treatment observed in our study (85 C for 2 min) would not be a practical treatment for ready-to-cook chickens to be sold retail because of the cooked appearance. It may, however, have some application for carcasses to be further processed, or that are to be presented to the consumer in a cooked condition, e.g. in institutions, restaurants, or "fast-food' outlets.

Acknowledgements

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Mention of specific brand names does not imply endorsement by the authors or institutions at which they are employed to the exclusion of others not mentioned.

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3-A SANITARY STANDARDS FOR SCRAPED SURFACE HEAT EXCHANGERS

Serial #31-00

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Scraped surface heat exchanger specifications heretofore or hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion, are equivalent or better may be submitted for the joint consideration of IAMFES, USPHS, and DIC at anytime.

A. *SCOPE*

A.1 These standards cover the sanitary aspects of scraped surface heat exchangers for adding heat to or removing heat from milk and milk products. These standards do not pertain to freezers for ice cream, ices and similarly frozen dairy foods[1] nor to batch processors for milk and milk products.[2]

A.2 In order to conform with these 3-A Sanitary Standards, scraped surface heat exchangers shall comply with the following design, material and fabrication criteria.

B. *DEFINITIONS*

B.1 *Scraped Surface Heat Exchanger*: (Referred to as SSHE throughout these 3-A Sanitary Standards) shall mean a cylinder(s) with closed ends, means for heating or cooling, having a precise wiping or scraping blade(s) for removing the heated or cooled product from the cylinder wall(s), and through which the product flows continuously under pressure.

B.2 *Product*: Shall mean milk and milk products.

B.3 *Surfaces*

B.3.1 *Product Contact Surfaces*: Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop or be drawn into the product.

B.3.2 *Non-Product Contact Surfaces*: Shall mean all other exposed surfaces.

B.4 *Mechanical Cleaning or Mechanically Cleaning*: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

B.5 *Engineering Plating*: Shall mean plated to specific dimensions or processed to specified dimensions after plating, and for these standards, the minimum thickness shall be 0.0002 inch for all product contact parts except as hereinafter specified.[3]

C. *MATERIALS*

C.1 All product contact surfaces shall be of stainless

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[1]Sanitary criteria for freezers will be found in the "3-A Sanitary Standards for Batch and Continuous Freezers for Ice Cream, Ices and Similarly Frozen Dairy Foods, Serial #1900" and amendments thereto.

[2]Sanitary criteria for batch processors will be found in the "3-A Sanitary Standards for Non-Coil Type Batch Processors for Milk and Milk Products, Serial #2500."

[3]QQ-C-320a Federal Specification for Chromium Plating (Electrodeposited) July 26, 1954. (Available from General Services Administration, Seventh and D Streets, NW, Room 1643, Washington, D.C.)

QQ-N-290 Federal Specification for Nickel Plating (Electrodeposited) April 5, 1954 and Amendment 1, December 13, 1961, (Available from General Services Administration, 7th & D Streets, NW, Room 1643, Washington, D.C.)

steel of the AISI 300 series[4] or corresponding ACI[5] types (See Appendix, Section E.), or metal that is non-toxic and non-absorbent, and which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types, except that:

C.1.1
Cylinders made of the materials provided for in C.1 may be covered with an engineering plating of chromium or an equally corrosion-resistant and wear-resistant non-toxic material.

C.1.2
Cylinders may also be made of other non-toxic structurally suitable heat-exchange metal made corrosion-resistant and wear-resistant by covering the product contact surface(s) with an engineering plating of chromium or an equally corrosion-resistant, non-toxic metal. When steel other than stainless steel is used, the minimum thickness of the engineering plating shall be 0.002 inch.

C.1.3
Cylinders, bearings, springs, shafts, couplings, drive and mounting pins, seal parts, and scraping parts may also be made of non-toxic hardenable, corrosion-resistant stainless metal (AISI 400 series stainless steel or equivalent) or these materials covered with an engineering plating of nickel, chromium or an equally corrosion-resistant, non-toxic metal.

C.1.4
Solder, when used, shall have a tin content of not less than 50% and the remainder shall be lead. It shall be cadmium free and non-absorbent.

C.1.5
Silver soldered or brazed areas and silver solder or braze material shall be non-toxic and corrosion-resistant.

C.1.6
Rubber and rubber-like materials may be used for gaskets, seals and parts used in similar applications. These materials shall conform to the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800."

C.1.7
Plastic materials may be used for bearings, scraping parts, gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000" as amended.

C.1.8
Where materials having certain inherent functional properties are required for specific applications, such as scraper parts and rotary seals, carbon and/or ceramic materials may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.9
Single-service sanitary type gaskets may be used.

C.2
All materials having a product contact surface(s) used in the construction of an SSHE designed to be used in a processing system to be sterilized by heat and operated at a temperature of 250°F or higher shall be such that they can be (1) sterilized by saturated steam or water under pressure at a temperature of at least 250°F and (2) operated at the temperature required for processng.

C.3
All non-product contact surfaces shall be of corrosion-resistant materials or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.
FABRICATION

D.1
All product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets. (See Appendix, Section F.)

D.2
All permanent joints in metallic product contact surfaces shall be welded. If it is impractical to weld, they may be silver soldered or brazed, or if this is not practical, the joint may be fitted in a manner that it will be completely rigid and without pockets or crevices. All such areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

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[4]The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from: American Iron and Steel Institute, 150 East 42nd Street, New York, NY 10017.

[5]Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, OH 44116.

D.3
Solder may be used around blade mounting pins, bushings and bearings for flushing joints and producng fillets for minimum radii.

D.4
All product contact surfaces of an SSHE not designed to be mechanically cleaned shall be easily accessible for cleaning and inspection either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.5
An SSHE that is to be mechanically cleaned shall be designed so that all product contact surfaces of the SSHE (1) can be mechanically cleaned and (2) are accessible for inspection.

D.6
All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch except that:

D.6.1
Where smaller radii are required for essential functional reasons such as sealing ring grooves, scraper blade mounting pins and parts used in similar applications.

D.6.2
The minimum radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.6.3
When for functional reasons the radius must be less than 1/32 inch, in such applications as flat sealing surfaces, the product contact surface of this internal angle must be readily accessible for cleaning and inspection.

D.7
All sanitary tubing, fittings and connections shall conform with the applicable provisions of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809," as amended and supplements thereto.

D.8
There shall be no threads on product contact surfaces.

D.9
Coil springs having product contact surfaces shall have at least 3/32 inch openings between coils including the ends when the spring is in a free position.

D.10
An SSHE designed to be used in a processing system to be sterilized with heat and operated at a temperature of 250°F or higher shall comply with the following criteria in addition to other criteria in this standard.

D.10.1
The construction shall be such that the SSHE can be (1) sterilized by saturated steam or water under pressure at a temperature of at least 250°F and (2) operated at the temperature required for processing.

D.10.2
The SSHE shall have a steam or other sterilizing medium chamber(s) surrounding the shaft(s) adjacent to the seal required by D.11.1.

D.10.3
The connection(s) on the steam or other sterilizing medium chamber(s) for the steam or other sterilizing medium lines shall be such that the lines can be securely fastened to the connection(s). The lines shall be connected in a manner that they may be disconnected to allow the sterilizing medium chamber to be inspected and cleaned if necessary.

D.11
The shaft(s) of an SSHE shall have a seal of a packless type, sanitary in design.

D.11.1
The seal(s) in an SSHE designed to be used in a processing system to be sterilized by heat and operated at a temperature of 250°F or higher shall be between the product contact surface and the steam or other sterilizing medium chamber.

D.12
The means of supporting an SSHE shall be one of the following:

D.12.1
With legs: Legs shall be smooth with rounded ends, have no exposed threads, and shall be of sufficient length to provide a clearance between the lowest part of the base and the floor of no less than six inches. Legs made of hollow stock shall be sealed.

D.12.2
Mounted on a slab or island: The base shall be designed for sealing to the slab or island surface. (See Appendix, Section G.)

D.12.3
Mounted on a wall or column: The point of attachment of an SSHE cylinder(s) to its mounting shall be designed for sealing. The mounting, if supplied by the SSHE manufacturer shall be designed for sealing to the wall or column. The design of

an SSHE with a vertical cylinder(s) to be mounted on a wall shall be such that there will be at least a 4-inch clearance between the outside of the cylinder(s) and the wall.

D.13
An SSHE designed to be installed partially outside a processing area, shall be provided with a plate or other suitable member to close the opening in the processing room wall or ceiling and shall be such that it can be sealed to the wall or ceiling.

D.14
The SSHE shall be designed so that there is at least a 4-inch space between the driving mechanism and the cylinder(s) when parts normally removed during cleaning have been removed.

D.15
Any guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection shall be designed so that it can be removed without the use of tools.

D.16
Non-product contact surfaces shall be free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

D.17
An SSHE shall have an information plate in juxtaposition to the name plate giving the following information or the information shall appear on the name plate:

(1) The maximum temperature and pressure at whch the SSHE can be operated.
(2) If the SSHE is or is not designed for mechanical cleaning.
(3) A statement that to prevent corrosion the recommendations of the SSHE manufacturer should be followed with respect to time, temperature and the concentration of specific cleaning solutions and chemical bactericides.

APPENDIX

E.
STAINLESS STEEL MATERIALS
Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel equivalent to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[6] Specifications A296-68 and A351-70.

F.
PRODUCT CONTACT SURFACE FINISH
Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.
SLABS OR ISLANDS
When an SSHE is designed to be installed on a slab or island, the dimensions of the slab or island should be such that the base of the SSHE will extend beyond the slab or island at least one inch in all horizontal directions. The slab or island should be of sufficient height so that the bottom of all product connections are not less than 4 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the SSHE base and the slab or island should be sealed.

These standards shall become effective Sept. 5, 1974.

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[6]Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa 19103.

CHANGES IN MILK, WHEY, AND BLUE CHEESE AS INDUCED BY BENZOYL PEROXIDE[1, 2]

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Abstract

Milk proteins were subjected to treatment with various levels of benzoyl peroxide, with and without heating at 60 C for 2 h. Heating had a pronounced effect on whey proteins, but polyacrylamide gel electrophoresis revealed changes in proteins not attributable to heat alone. The effect on proteins was reflected in an increased tendency for the benzoyl peroxide-heat treated cheeses to expel moisture during leakage tests. Use of 17.8 ppm benzoyl peroxide resulted in a markedly whiter cheese than that made using 5.9 ppm and reflectance studies indicated this to be true even when no heat treatment accompanied the benzoyl peroxide. Use of benzoyl peroxide in the bleaching process did not decrease mold development in ripening loaves nor was acid production by lactic cultures diminished. In addition, proteolysis of milk proteins by rennet was not reduced by the presence of benzoyl peroxide.

Benzoyl peroxide is approved for use as a bleaching agent in the manufacture of various cheeses *(7)*. Kuramoto and Jezeski *(17)* studied the influence of time, temperature, and concentration on the bleaching of carotenoid pigments in (Blue) cheese milk by benzoyl peroxide. They reported that .0009% (9 ppm) at bleaching temperatures of 51.7 and 62.8 C gave the best results when flavor and carotenoid destruction were used as the criteria. Carotenoid destruction was much faster, however, at 74 C. Use of .0018% (18 ppm) resulted in oxidized and tallowy flavors, and a lower concentration of .00045% (4.5 ppm) produced ineffective bleaching. Pedersen et al. *(24)*, in a recent study of the leakage defect of Blue cheese, used a bleaching concentration of .0154% (154 ppm) in the cream which, if undissipated, would have resulted in a final concentration of .00059% (5.9 ppm) in the vat milk. They reported a correlation between use of benzoyl peroxide as the whitening agent and occurrence of the leakage defect. An adverse effect of the stretching and melting qualities of Mozzarella cheese by benzoyl peroxide also has been observed *(16)*.

Other than the report by Hunziker *(13)* on effects of benzoyl peroxide on cream for butter making, very little information is available about its use in the dairy industry *(17)*. Considerable information is available, however, with regard to its use in the bleaching of flour and as a component in various pharmaceutical preparations *(1, 11, 15, 28)*. Benzoyl peroxide also has been reported to inhibit germination of bacterial spores *(27)*. The present study investigates the effects of benzoyl peroxide on the casein and whey proteins of milk as they relate to the manufacture of Blue cheese.

Materials and Methods

Benzoyl peroxide

Benzoyl peroxide used in this investigation was obtained from Cadet Chemical Corp., Burt, N.Y., and was labelled 99% pure.

Blue cheese manufacture

The procedure of Pedersen et al. *(24)* was used with some modifications. Three lots of four vats each were manufactured. Milk was obtained commercially and was separated at 3.3 C. The raw cream was subjected to various treatments (Table 1), brought to 37.7 C, and homogenized at 105.5 kg/cm^2. Portions of cream were mixed with raw skimmilk in small pilot-plant vats to yield milkfat contents of 3.8%. Calcium chloride was added to each vat at 7.0 g/45.4 kg of milk. Veal rennet (6.0 ml/45.4 kg) was added when the titratable acidity calculated as lactic acid measured .26%.

At the time of dipping, approximately 1% salt and .05% mold powder were added to the curd before hooping. But, two hoops of curd from each of the vats in Trial II received no mold powder and were used in electrophoretic and color-comparison studies. The curd for one hoop from each of the vats in lot I was dipped in a whey culture of *Streptococcus faecalis* var. *liquefaciens* to determine the effect of a proteolytic microorganism on the leakage defect.

All cheeses remained uncovered throughout the first 30 days of curing and were then coated with paraffin.

A sample of milk taken from each vat just before culture addition and a sample of whey taken at the time of hooping were quickly refrigerated and saved for electrophoresis.

Leakage test

Determination of the volume of exudate from cut cheese loaves at 21 C was done according to the method of Pedersen et al. *(24)*.

Polyacrylamide gel electrophoresis

Procedures used were those described by Maizel *(19)*. A

[1]Journal Paper No. J-7667 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1839.
[2]This investigation was supported by a grant from Universal Foods Corp., Milwaukee, Wisconsin 53201.
[3]Present address: Tolibia Cheese, Incorporated, 45 E. Scott Street, Fond du Lac, Wisconsin 54935.
[4]Present address: Microlife Technics, Sarasota, Florida 33580.

TABLE 1. TREATMENT OF PASTEURIZED CREAM USED TO MANUFACTURE BLUE CHEESE

	Vat No.	Treatment of cream[1]	Whitener
Trial I	1	None	Blego
	2	5.9 ppm benzoyl peroxide + heat[2]	Benzoyl peroxide
	3	Heat alone	Blego
	4	5.9 ppm benzoyl peroxide—no heat	Benzoyl peroxide
Trial II	1	None	None
	2	None	Blego
	3	17.8 ppm benzoyl peroxide + heat	Benzoyl peroxide
	4	17.8 ppm benzoyl peroxide—no heat	Benzoyl peroxide

[1]All cream was homogenized at 37.7 C.
[2]Brought to 60 C, held for 2 h, and cooled to 37.7 C.

Canalco Model 12 Apparatus (Canal Industrial Corp., Rockville, Maryland) was used. A 7% gel was prepared, and samples were introduced either by direct layering in 10% sucrose or by gelling into dilute stacking gel. Electrophoresis was done at 1.5 ma/tube until the tracking dye entered the separating gel, and the process was completed at 3.5 ma/tube. Protein bands were stained in amido black in 7.5% acetic acid for 2 to 12 h. The gels were then destained electrophoretically at 6 ma/tube.

Activity tests

The acid-producing activity of nine different lactic cultures was determined in MATRIX medium (Galloway-West Co., Fond du Lac, Wisconsin) and whole milk containing 17.8, 100, 1,000, and 10,000 ppm benzoyl peroxide, heated and unheated. The activity tests were done by the procedure of Reif *(25)*. Standard Plate Counts were determined in Eugonagar (BBL, Cockeysville, Maryland) immediately after inoculation of starter organisms into milk and after 6 h of incubation.

Sample preparation

Whole casein was prepared by the method of Fox and Kosikowski *(8)*; whey was prepared by acidification and centrifugation of raw skimmilk. Bovine serum albumin (BSA) was obtained from Pentex, Inc., Kankakee, Illinois.

Effect of benzoyl peroxide treatment on proteolysis

Samples of raw skimmilk and whole casein were treated with various levels of benzoyl peroxide, and the extent of proteolysis by rennet was determined by measuring ninhydrin-reactive compounds *(21)*.

Reflectance

The reflectance spectra of mold-free cheeses were determined using the reflectance attachment on a Beckman DK-2A recording spectrophotometer.

Cultures

Two mixed-strain, multiple-type commercial cheese starters (A and B), two strains of *Streptococcus cremoris* (SC 223 and HP), two strains of *Lactobacillus bulgaricus* (LB1 and SUC), *Streptococcus lactis* 7962 and *Streptococcus diacetilactis* 18-16, used in this investigation, were taken from the culture collection of the Department of Food Technology, Iowa State University. Only commercial culture A was used in the manufacture of the Blue cheese.

Solubility

The solubility of benzoyl peroxide in water, whey, castor oil, soybean oil, mineral oil, butter, and milk was determined benzoyl peroxide to duplicate tubes and 10% benzoyl peroxide to the two remaining tubes. For each concentration, one of the duplicate tubes and its contents was heated for 2 h at 60 C and cooled.

RESULTS

Leakage and color

Leakage tests were done on two cheese loaves from each of the four vats in Trials I and II. The average leakage loss per loaf from vats 1 through 4 in Trial I was 19, 59, 27, and 21 ml, respectively. This supports the work of Pedersen et al. *(24)* correlating the use of benzoyl peroxide-heat treatment with the leakage problem.

Cheese loaves made from curd dipped in a whey culture of *S. faecalis* var. *liquefaciens* did not increase their tendency toward leakage at 60 days. Indeed, these loaves were more firm than other loaves from the same vat. Extension of the ripening period, however, resulted in a soft and very bitter cheese.

Visual examination of loaves from Trial I did not reveal any increase in the degree of whiteness as a result of treatment with benzoyl peroxide at the rate of 7 g/45.4 kg milk fat (5.9 ppm). This was true whether or not benzoyl peroxide was accompanied by heating for 2 h at 60 C. A threefold increase in benzoyl peroxide (Trial II), however, resulted in a markedly whiter cheese, independent of the heat treatment.

Results of the reflectance spectra (Fig. 1) on the mold-free cheese loaves in Trial II indicated a dominant wavelength of 410 to 530 nm in cheese from all four vats. The reflectance tracings for cheese whitened by Blego (Chr. Hansen's Laboratory, Inc., Milwaukee, Wisconsin), a masking agent, and that for cheese receiving no whitening treatment were nearly identical. Similar tracings for the cheese pairs treated with benzoyl peroxide (17.8 ppm) were nearly identical, but different from the untreated and Blego-treated cheeses. The two samples treated with benzoyl peroxide, heated and unheated, had a markedly higher reflectance than the untreated cheeses. The reflectance at 470 nm for cheese treated with benzoyl peroxide was 70%, and the value for untreated cheese was 62%.

Activities

The acid-producing activity and the viable population of various lactic cultures in MATRIX medium and in whole milk were not decreased by the presence of benzoyl peroxide in concentrations as high

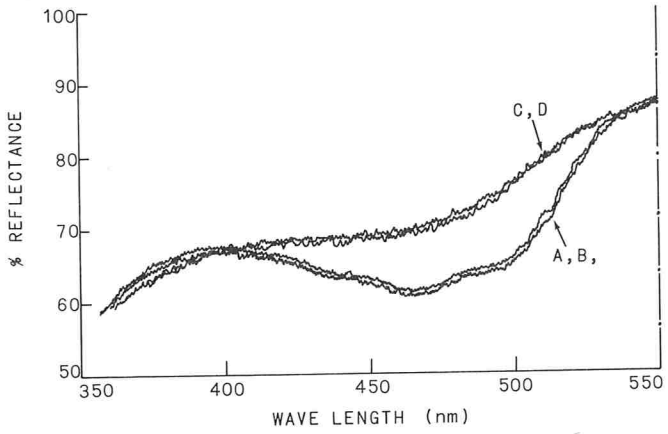

Figure 1. Effect of benzoyl peroxide on reflectance spectra of mold-free cheeses. A. No milk treatment. B. No benzoyl peroxide, Blego added. C. 17.8 ppm benzoyl peroxide, 2 h at 60 C. D. 17.8 ppm benzoyl peroxide, no heat.

as 10,000 ppm. This supports the earlier finding of Swiatek and Poznański *(30)* that lactose fermentation in cheese milk was not decreased by addition of 9 ppm benzoyl peroxide. There was a difference between acid-producing activity of some cultures in heated and unheated milk independent of the benzoyl peroxide treatment. For example, the activity test for starter culture A was .56% (expressed as percent lactic acid) in heated milk, with and without added benzoyl peroxide, but the corresponding activity in unheated milk was .62%. Such differences have been investigated by Gilliland *(9)*.

Proteolysis

There was no obvious change in the rate of proteolysis of skimmilk or washed casein by rennet when treated with benzoyl peroxide at .001% to 5%. This was evident by the lack of appreciable differences between the absorbency of treated and untreated samples. Use of hydrogen peroxide has been reported to increase the susceptibility of milk proteins to proteolysis *(6, 8)*.

Electrophoresis

Polyacrylamide gel electrophoresis patterns for whey, casein, and bovine serum albumin are shown in Fig. 2, 3, 4, and 5. The patterns obtained by Melachouris *(20)* and Vedamuthu et al. *(32)* were used as identification references.

The benzoyl peroxide-heat bleaching treatment

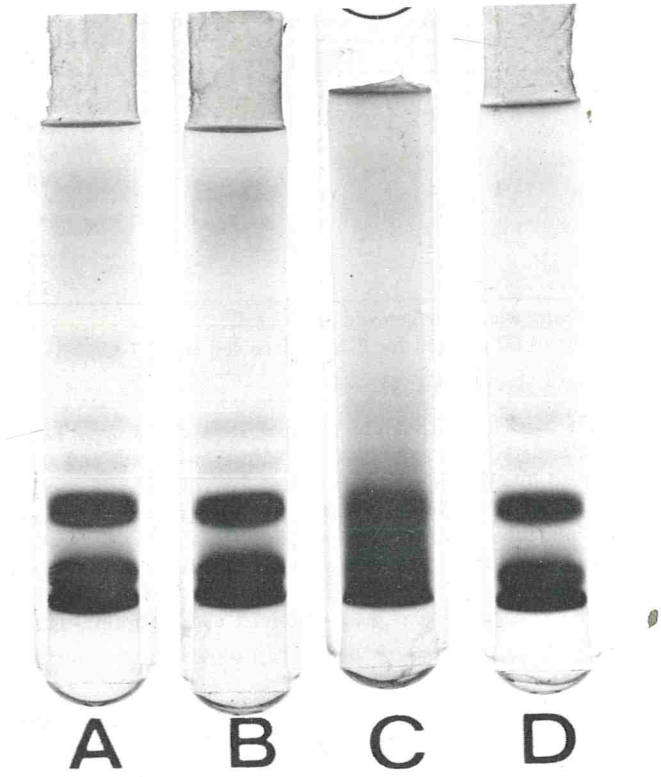

Figure 2. Effect of benzoyl peroxide on polyacrylamide gel electrophoretic patterns of whey proteins. A. Untreated whey control. B. 1% benzoyl peroxide, unheated. C. 1% benzoyl peroxide, heated 2 h at 60 C. D. No benzoyl peroxide, heated 2 h at 60 C.

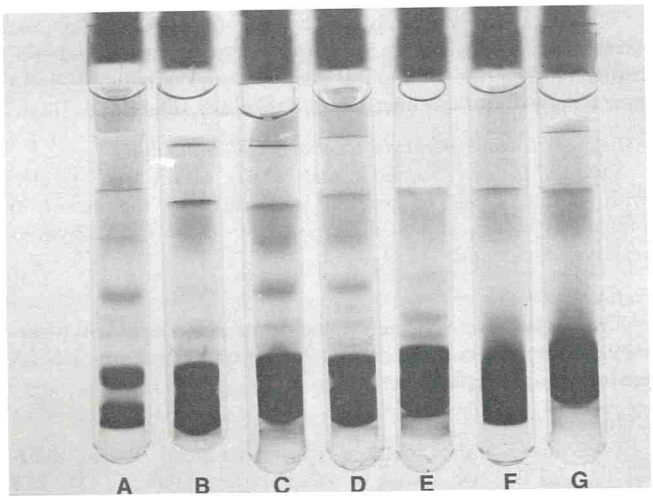

Figure 3. Effect of benzoyl peroxide on disc gel electrophoretic patterns of whey proteins. A. Untreated whey control. B. No benzoyl peroxide, heated 2 h at 60 C. C. 1% benzoyl peroxide, unheated. D. .01% benzoyl peroxide, heated. E. .1% benzoyl peroxide, heated. F. 1% benzoyl peroxide, heated. G. 5% benzoyl peroxide, heated. (Samples B and D through G were heated 2 h at 60 C).

produced some distinct changes in the pattern for whey proteins (Fig. 2). The β-lactoglobulin and α-lactalbumin bands became nondiscreet and appeared as one wide band with some trailing compon-

ents, as evidenced by the smear behind the band. In addition, the serum albumin and proteose peptone bands disappeared or became diffuse, as did the immune globulin bands. These changes in whey proteins were dependent on the benzoyl peroxide concentration and heat treatment. Figure 3 indicates, with concentrated whey, that changes were most evident when 1% or 5% benzoyl peroxide was used.

Comparison of the electrophoretic pattern of untreated milk with the pattern of vat milk just before culture addition revealed no differences. Similarly patterns for untreated whey and for vat whey at the time of hooping were seemingly identical.

Patterns in Fig. 4 show that 1% benzoyl peroxide, either with or without the heat treatment, causes some changes in the protein structure of bovine serum albumin. The bands tend to be less distinct, as with β-lactoglobulin, when heat treatment accompanied addition of benzoyl peroxide, and additional bands seem to result from the benzoyl peroxide treatment.

The effect on casein is much less pronounced (Fig. 5); although the benzoyl peroxide-heat treated sample produced much more smearing between major bands.

Solubility

The solubility of 10% benzoyl peroxide in water, whey, soybean oil, and castor oil is shown in Fig. 6. Even after heating 2 h at 60 C, undissolved benzoyl peroxide was still present in water, whey, and in milk (although not shown). Benzoyl peroxide was soluble to a greater extent in soybean oil, castor oil, and butter before heating, but dissolved completely in less than 30 min at 60 C. In contrast, benzoyl peroxide did not dissolve in mineral oil even after 2 h at 60 C.

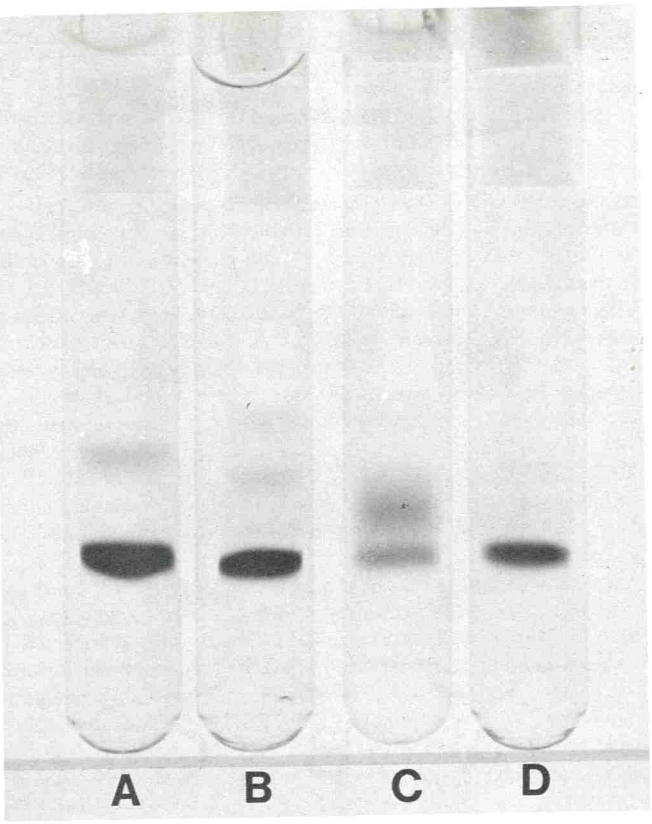

Figure 4. Effect of benzoyl peroxide on polyacrylamide gel electrophoretic patterns of bovine serum albumin (BSA). A. Untreated .15% BSA control. B. 1% benzoyl peroxide, unheated. C. 1% benzoyl peroxide, heated 2 h at 60 C. D. No benzoyl peroxide, heated 2 h at 60 C.

DISCUSSION

It has been demonstrated in this study as well as in that by Pedersen et al. *(24)* that the benzoyl peroxide-heat treatment of cream for Blue cheese increases the tendency for leakage as the temperature of the cheese increases, as may happen to cheese in transit. Water binding properties of cheese must be associated with protein, most of which is casein. It is this protein matrix that must ultimately be altered. Protein changes could result from heat alone *(12, 22)*, from action of benzoyl peroxide on protein, or from formation of lipid peroxides which, in turn, could oxidize protein thiols *(4, 14, 18, 23, 26, 31)*. The latter possibility must be given consideration because only the lipid portion of the vat milk, with just a small portion of protein, is subjected to the bleaching process at 60 C and because of the findings that benzoyl peroxide is highly soluble in milkfat. The thermally dependent reaction rate of Kuramoto and Jezeski *(17)* would tend to support lipid involvement.

Benzoyl peroxide was not readily soluble in whole milk, and a residue remained after heating for 2 h at 60 C. Should this occur during cheese manufacture, unreacted benzoyl peroxide would be trapped and concentrated in curd. It would then be present to react with proteins or lipids during curing. Kuramoto and Jezeski *(17)* reported a greater bleaching effect on cream with a higher fat content and suggested that benzoyl peroxide remains suspended in the skimmilk portion and increases in relative concentration as the fat percentage increases. Failure of the heat treatment to destroy benzoyl peroxide could leave it present in the cheese to be reactive during curing.

The stability of benzoyl peroxide to heat was amply demonstrated by Gruber and Klein *(11)* when the benzoyl peroxide powder was held 8 weeks at 60 C with $< 5\%$ loss of potency and $< 30\%$ loss in a pharmaceutical lotion formulation held 6.5 weeks at 46 C. In addition, benzoyl peroxide has been shown to cause skin irritation *(5, 29)*. These cases of

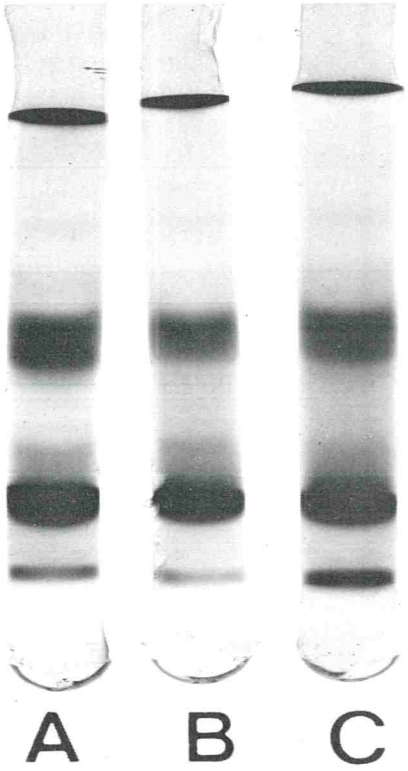

Figure 5. Effect of 1% benzoyl peroxide on polyacrylamide gel patterns of casein. A. Untreated casein control. B. Benzoyl peroxide. C. Benzoyl peroxide, heated 2 h at 60 C.

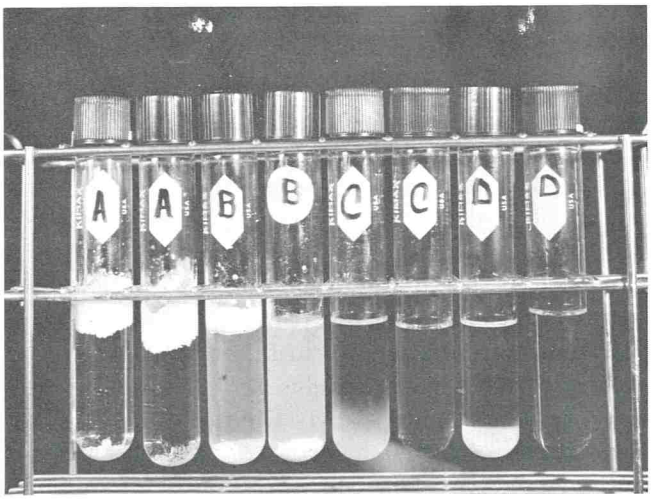

Figure 6. Effect of medium and heat on solubility of 10% benzoyl peroxide. Sample on right of each pair was heated 2 h at 60 C. A. Water. B. Whey. C. Soybean oil. D. Castor oil.

dermatitis, however, have been attributable to concentrations several hundred times greater than the legal maximum for cheese. The concern generated by the non-destruction of benzoyl peroxide in whey might be alleviated by the solubility results in lipids, as well as by the reports of Arnold *(1)* and Sharratt et al. *(28)* that no ill effects, or carcinogenic hazards, exist with consumption of extremely high levels of the bleaching agent.

Kuramoto and Jezeski *(17)* reported that, whenever 18 ppm benzoyl peroxide was used in the bleaching process, the cheese developed undesirable oxidized and tallowy flavors. This present study found such flavor defects barely discernible in benzoyl peroxide-heat treated cheese and absent in cheese made from cream bleached with 17.8 ppm benzoyl peroxide with no accompanying heat treatment.

It was possible, by polyacrylamide gel electrophoresis, to demonstrate changes in milk proteins as a result of treatment with benzoyl peroxide after 2 h at 60 C. The effect on casein, although visibly less pronounced, may be more important than the effect on the whey proteins. The combination of heat and benzoyl peroxide was much more disruptive to the electrophoretic patterns than was either alone. These induced changes could give rise to interactions between casein-complex components and the denatured whey proteins, or, perhaps, a more hydrated protein structure could result from various interactions. Changes in milk and whey proteins as a result of the action of added hydrogen peroxide have been reported previously *(3, 8, 10)*.

In summary, benzoyl peroxide at high levels does cause changes in milk proteins that might be evidenced at lower levels by the resultant increased tendency for exudate loss from Blue cheese. The heat treatment, however, increases the protein denaturation markedly. This, coupled with the failure of heat to increase the whiteness of cheese treated with 17.8 ppm benzoyl peroxide and the failure of 2 h at 60 C to destroy benzoyl peroxide in whey casts doubt on the necessity of the heat treatment. The difficulty in dispersing benzoyl peroxide in milk led to concern about its solubility in cream and its possible retention in curd where it could affect starter culture and mold development. This was of special interest in view of the reported effects of hydrogen peroxide on the growth of *Pseudomonas fragi (2)*. Careful observation of cheese loaves during ripening did not indicate any inhibition of mold growth by benzoyl peroxide treatment. Starter culture fermentation was unaffected by high (5%) concentrations of benzoyl peroxide. No ill effects have been attributed to consumption of benzoyl peroxide. This suggests the possibility of using 15-20 ppm benzoyl and eliminating the costly and time-consuming heat treatment, which seems more harmful than beneficial. Legal residuals should cause neither a health hazard nor an alteration in the ripening events. Perhaps new consideration needs to be given to the time, temperature, and concentration involved in the ben-

zoyl peroxide-heat treatment currently used by some Blue cheese manufacturers.

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COMMERCIAL STERILIZATION AND ASEPTIC PACKAGING OF MILK PRODUCTS

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ABSTRACT

Milk is nature's most nearly perfect food and to utilize its nutritive values milk must be free from all pathogenic microorganisms. Pathogenic bacteria present, if any, in raw milk supplies are destroyed by the pasteurization process. Milk and its fluid products, so treated, possess on an average of 7-10 days shelflife, which in modern sophisticated distribution systems, is not adequate. Commercial sterilization in conjunction with aseptic packaging extend the shelflife of milk products to 90+ days. These products, excellent in performance, are not yet popular in the dairy case but, if consistent efforts are continued for their promotion, will win consumer's confidence. Increasing application of the sterilization process in the dairy industry has prompted marketing of numerous types and brands of sterlilizing equipment. Every sterilization operation, due to differences in equipment and range of products manufactured, is faced with various problems in commercial applications of the process. Besides technical problems, label declaration and mode of distribution of these products cause legal complications in many areas of this country. Furthermore, no definition for commercially sterilized milk products aseptically packaged in flexible containers has been officially established by federal, state, and local regulatory agencies. Regardless of problems and challenges in commercial application of the sterilization process in the milk industry, potential opportunities in this country for milk products, thus processed, are great.

INTRODUCTION

Milk is the most nearly perfect food but one of the most perishable. Microorganisms can cause foods in general and milk in particular to deteriorate. Therefore, it is necessary to destroy the organisms in milk to safeguard its healthfulness and prolong its shelflife, palatability, and wholesomeness. Application of heat is the most common method to destroy microorganisms in foods. With milk, destruction of any pathogenic organism that may be present is achieved by pasteurization which consists of heating the milk, holding it at a specific temperature, and subsequent cooling of the product.

There is no denying that the concept of pasteurization is excellent. Unfortunately, the process is not adequate for present-day merchandising of milk products. A recent study at the University of Florida *(1)* revealed that leaving regular pasteurized milk in a shopper's hot automobile encouraged growth of microorganisms that survived the pasteurization process or were present as post-pasteurization contaminants and thus considerably reduced subsequent shelflife. Even with refrigeration, certain microorganisms continue to grow, and consequently, milk products often develop undesirable characteristics with the lapse of time.

The average shelflife of pasteurized milk and its fluid products is about 7 to 10 days. Depending on the distribution area, 1 or 2 days elapse before the product is available to consumers. Nevertheless, this 7 to 10 days of shelflife may be more than adequate for regular pasteurized milk because of its high turnover at the retail level. But a 7-day shelflife often is not enough for products such as cream for whipping, coffee cream, and half and half because of their slower turnover.

Consequently, another method of processing to extend shelflife has been adopted by several dairy processors. Absolute sterility in a large volume of milk product is practically impossible. This is because the severe heating needed to achieve absolute sterility could profoundly alter the nutritive and organoleptic properties and render the product unfit for human consumption. Hence, the term sterilization as used in this paper refers to commercial sterilization. Most milk products are sterilized at temperatures between 275 and 300 F for 2 to 8 sec but they may still contain viable spores which normally don't grow to cause any subsequent spoilage of the product. Accordingly, a sterilized milk product need not be sterile to achieve a longer shelflife. These products, of course, should be free of harmful or proliferating organisms, and keep without deterioration for at least 30 days. However, with a proper combination of efficient sterilization and aseptic packaging systems, a shelflife of 90+ days can be achieved in milk products.

PROCESSING METHODS

Commercial sterilization of fluid milk products has recently been accepted in America. Several food manufacturers are focussing their efforts in this direction to meet the growing demand in the marketplace for such milk products. In recognition of this situation, several equipment manufacturers have de-

[1]Present address: Ohio Processors, Inc., London, Ohio 43140

veloped myriads of sterilization equipment and aseptic packaging machines.

Sterilization systems

A wide variety (sizes, shapes, and designs) of equipment is available for commercial sterilization of milk products. There are direct and indirect heating techniques and each imparts different characteristics to fluid milk products. Factors that should govern selection of a heating system include: *(a)* product considerations, *(b)* mechanical considerations, *(c)* automation, *(d)* economics, *(e)* legal considerations, and *(f)* correlation with packaging system.

Direct heating methods. *(a)* Steam injection technique. In this steam-into-milk process, sterilization is achieved by injecting high pressure steam into a line of constantly flowing product. The added steam is removed in a sterile vacuum chamber. This is also called "Uperization." *(b)* Steam infusion technique. In this milk-into-steam process the product is atomized into a pressurized steam chamber, then transferred into a sterile vacuum chamber where excess steam is removed.

In these direct heating systems the product is heated and cooled rapidly and is also deodorized in the evaporative cooling section. There is little product deposited on equipment surfaces because usually no metal surface is hotter than the product itself, and surfaces where deposits are likely to form are smaller because of the short time between heating and cooling. Thus, the chemical and physical changes in the product are less pronounced and natural color, flavor, and essential nutrients are preserved.

Direct heating methods are illegal in some countries (e.g. United Kingdom) as it is feared that impurities from steam may be carried into milk. Where sanctioned, the steam supply must be of culinary quality and the quantity of water before and after heating must be carefully controlled and maintained exactly.

These systems, however, are designed for both thin fluid products (e.g. coffee and whipping creams, half and half) and highly viscous products such as puddings but are best suited for extremely heat sensitive products like fortified skim milk.

Indirect heating methods. *(a)* Plate system. Most systems are similar in shape to the common pasteurizing press but different in design and capacity. The equipment consists of corrugated plates of stainless steel, joined in such a way that extremely thin layers of product are constantly in contact with the heat exchange surface. Because of this design feature relatively low pressure exists in the heat exchanger which means relatively low velocities and relatively low turbulence. This process is suitable for sterilizing all fluid milk products.

(b) Tubular system. This seamless tubular heat exchange system can withstand high pressure. The product flows at high velocity and results in extremely high heat transfer rates. Since high product pressure and turbulence virtually eliminate burn-on of product, this system is ideal for processing products without added emulsifiers or stabilizers and is, therefore, preferred for sterilization of half and half, coffee cream, whipping cream, etc.

(c) Scraped surface system. This system is designed to sterilize high viscosity products with or without particles, by continually scraping product off the heat exchange surface. Puddings and ice cream mixes can be easily and continuously sterilized in this system. Regeneration is not included hence, higher operation cost.

Aseptic packaging system

Packaging of sterilized milk products under aseptic conditions is essential for extending shelflife. The locked-in cooked flavor in metal cans and light-induced changes in glass containers of sterilized products stimulated research and development of a packaging system which could employ a flexible paper laminate container for packaging of such products. In early 1960 Tetra-Pak of Sweden introduced an aseptic packaging system which utilizes paper foil laminate to form a tetrahedron-shaped flexible container. This system has drawbacks such as size limitations and machine speed, and the containers also present problems to processors and consumers. As a result, the Ex-Cell-O corporation, after 5 years of study, planning, and fabrication, put into operation in 1968 an aseptic packaging system which uses comparatively rigid containers to package fluid milk products. The new machine is designated as the Pure-Pak NLL and its use assures extended shelflife for all sterilized products. This compact system forms, fills, seals, and codes 45 aluminum foil lined paper containers per minute in sizes from ½ pint through the quart.

Special features of this aseptic Pure-Pak packaging system are: *(a)* sterilized multifoil container blanks are loaded conventionally and bottom sealed, *(b)* bottom sealed containers are resterilized by chemical fogging, *(c)* containers are dried with hot sterile air, *(d)* containers are filled and sealed in a sterile air atmosphere, *(e)* a vacuum defoamer removes excess foam and helps secure an adequate top seal, and *(f)* individual containers are coded.

COMMERCIAL APPLICATIONS AND ASSOCIATED PROBLEMS

Nothing is perfect in this world including man and his creations. Sterilization and aseptic packaging of milk products are no exceptions to this axiom. These

processes usually work very well for dairy processors. However, consumers sometimes become disenchanted with sterilized milk products when they fail to live up to their expectations. Such complaints are generally caused by imperfections in the processing and packaging techniques. Imperfections oftentimes become inevitable when new processing techniques are applied to milk—one of nature's most perishable foods.

Processors in the United States guarantee that sterilized milk products have a shelflife of 6 weeks, 3 months, and longer. It is not uncommon to find containers from the same batch of sterilized product which 'spoil,' prematurely whereas others stay fresh and are palatable far beyond their expiration dates. Some of the variables of vital importance in sterilization and aseptic packaging processes of milk products are described in the following discussion.

Selection of raw material

Not all types of raw milk, milk powder, and cream are suitable as ingredients for milk products to be sterilized. Suppose a raw fluid milk product contains 1,000 spores/ml, and is heated with a resultant sterilizing effect of 10 (spores reduced through 10 log cycles). The end product would have one spore in every 10,000 quarts produced. Consequently, if the raw product had only 10 spores/ml, and was processed in the same manner it would have only one surviving spore in one million quarts.

The first requisite in producing high quality sterilized products is to exercise care so only raw material with initial high quality is selected. This minimizes problems caused by bacterial, chemical, and physical deterioration. Hence, initial quality of raw products will determine the quality of finished sterilized milk products. The sterilization process, therefore, is more or less comparable to the process of dry cleaning. The more soiled the clothes, the less efficient the process.

Selection of sterilization temperature

In addition to initial bacterial load, especially spores, in milk products the number of organisms surviving sterilization depends on the sterilizing efficiency of sterilizers. A sterilizing effect of 10 at 295 F for 2 sec is considered adequate for most milk products. Nevertheless, the nature and properties of milk products (viscosity, solid contents, heat sensitivity, etc.) must be considered for satisfactory time-temperature determination in the sterilization process.

Selection of homogenization sequence

Homogenization of sterilized milk products containing fat is essential to protect stability of the emulsion during subsequent storage. Determination of homogenizing pressure is predicated by the characteristics desired in products. In general, homogenization before sterilization results in a coarse texture and increased sedimentation in products. Also, homogenization of heavy cream before sterilization often results in large fat globules and clusters, and increased gel structure. Effects of homogenization before sterilization are more pronounced when direct heating systems are used.

Homogenization after sterilization creates the need for an aseptic homogenizer or homogenizing valve to prevent post-sterilization contamination of products. Homogenization after sterilization avoids the emulsion destabilizing effects of the heat treatment, helps to obtain proper division and dispersion of fat globules, and reduces protein destabilization. Homogenization after sterilization is also needed to avoid 'oiling off' caused by steam employed in direct heating of coffee and whipping creams.

Double homogenization (before and after sterilization) yields products (i.e. coffee cream, and half and half) that are less stable in hot coffee than those homogenized only after sterilization.

Selection of vacuum deaeration sequence

The flavor of sterilized milk products has been described as cooked or 'cabbage.' Cooked flavor in products following sterilization is related to liberation of volatile sulfur compounds or free sulfhydryl groups. This defect can be reduced significantly by deaerating products at about 170 F during the sterilization process.

Deaeration of milk products, before sterilization, removes the occluded gas together with some of the volatile sulfhydryl compounds which may have been produced during regenerative heating. Deaeration at this stage provides residence time at an elevated temperature to heat-stabilize the product being processed and reduces its oxygen content which prevents further liberation of cooked flavor producing compounds during final sterilization. A level of 6 ppm oxygen is desirable to preserve optimum flavor in milk products.

Deaeration after sterilization removes all gases in addition to any sulfhydryl compounds produced at this stage. This imparts an excellent flavor to fortified skim milk.

An operating pressure on the vacuum deaerator should be maintained between 8-10 inches of mercury for all milk products except for those that are flavored artificially, in which case it should be kept below 4 inches. Under no circumstances should the pressure exceed 15 inches because any pressure above this limit would dehydrate the product.

Cooling of products before packaging

Cooling of milk products after final sterilization is

necessary to prevent chemical, physical, and microbiological changes during subsequent storage of these products. Most low fat fluid milk products up to 70 F can be packaged aseptically in paper containers without a problem. However, half and half, and coffee and whipping creams must be packaged at 40 F or below to eliminate fat layer or plug formation induced by slow cooling of these products in containers during storage in the cold room. Homogenization helps to alleviate this problem to some extent but these products may be stored for weeks before becoming available to the consumers. Some stabilizers and emulsifiers also could be helpful but in certain areas, New York, for example, such additives are not permitted in fluid milk products.

Pure-Pak machine and containers

Sterilization along with aseptic packaging is a very sophisticated operation. Sterilized milk products must be packaged in containers that will maintain 'sterility' to extend their shelflife. Light-induced changes in the sterilized products packaged in glass jars, and cooked flavors locked in the canned products, have caused multifoil laminated paper containers to become popular for packaging of these products.

Pure-Pak containers are most commonly employed in the U.S.A. for aseptic packaging of sterilized products. The standard Ex-Cell-O aseptic filler packages 45 units, whereas some of the modified fillers can handle as many as 75 units/min. Misformed or inadequately sealed containers may create spoilage problems because at high filler speed it is impossible for a machine operator to check each container. During operation of the filler, a partial or complete clogging of the peroxide spray nozzle results in failure to chemically sterilize the product contact surface of the container and so may cause spoilage of product. However, a signal device which alerts the operator to the malfunction of the peroxide spray nozzle during operation of the machine can remedy this problem.

Container blanks can be another potential problem. Those that are tightly packed in cartons are usually damaged (bent) and could represent as much as 10% of the total. If an operator attempts to utilize these containers after correcting them, the bottom will not be formed properly in 9 out of 10 instances. When misformed, these containers may not get a uniform peroxide spray or may get stuck and stop the Pure-Pak aseptic packaging machine. Each time the machine stops, at least four filled containers under the top heater will not be sealed, thus resulting in loss and waste.

Industry feels that much improvement is desired in packing blanks to eliminate 'troubleshooting' for damaged containers. Reinforcement and/or cushioning of corners of cartons may help to protect containers during transportation and storage. It is advantageous to discard damaged blanks rather than use them.

The fifth panel (vertical seam) of the container could also use some technological improvements. Milk products with low viscosity or high sugar content will 'wick' (seam leak) even during cold storage and can potentially jeopardize the entire batch of sterilized product. It is believed that skiived edging (foil folded seam ends) of the fifth panel may be instrumental in curing the 'wicking' problem. Skiived edged containers are relatively expensive but it could pay dividends in the long run by eliminating the 'wicking' problem.

Testing sterility of finished products

Highly heat resistant bacteria surviving in a large volume of sterilized milk product can be a potential nemesis for a processor. So can any contamination picked up by a product in the packaging station. When a very small number of bacteria live in a large volume of product without growing (one bacterium in 10,000 or one million quarts of product) detection of bacterial metabolic products is practically impossible. Therefore, a sample containing living bacteria could be declared sterile because it would be impossible to determine presence of bacteria in a large volume of product.

When a large volume of product is distributed into individual containers a few of the spores that are not destroyed by the sterilization process may be introduced into a very few containers while all other containers are free of the spores. Results from examination of these containers would be valid only for the sample tested and not for the entire contents of the container. In addition, results might be valid only for the container tested and not for the entire batch.

The most common method to ascertain if a product is sterile is to test it bacteriologically. The Standard Plate Count and incubation at 90 F of finished products are being investigated in various parts of the country as suitable methods for laboratory control and more experience is needed before any national consensus on testing techniques is established.

Loading and unloading of finished products

In addition to careful processing and packaging, proper loading and unloading of sterilized milk products are essential to prevent any damage that could introduce contamination into products at this point. Sterilized products must be carefully placed in the truck. Cases of products must be palletized so as not to damage the gable top of the containers. Excessive agitation of product resulting from bumpy roads

might cause churning of fat in high fat content milk products. Failure to exercise care and responsibility during loading, transport, and unloading of these products could cause several problems with a resultant high loss.

Shoppers and sterilized products

An American housewife is considered to be the most sophisticated shopper in the world. Besides freshness and wholesomeness, present-day shoppers are demanding extended shelflife milk products. Consequently, milk processors in America are attempting to achieve this goal by means of sterilization and aseptic packaging operations.

But shoppers seem to hesitate to pick up a container of sterilized product from the dairy case because they are seemingly not as familiar with the meaning of the word "sterilized" as they are with "pasteurized." A majority of shoppers assume that something is either added to or removed from these products. It may be helpful for the processors to label these products "long life" rather than sterilized.

Consideration for legal status

The process of sterilization consists of applying thermal conditions as severe as possible while remaining within a range compatible with conservation of desirable organoleptic properties of milk products. National and international legal aspects must be taken into consideration to comply with requirements for processing, packaging, distributing, and marketing of sterilized milk products.

In the United Kingdom, ultra-high-temperature (UHT) milk is defined as milk which has been heat treated to not $<$ 270 F and held for not $<$ 1 sec. In West Germany, UHT milk is defined as milk that must be heated to a temperature between 275 and 300 F in approved equipment. The required holding time must be determined by an official test. Swedish Food Ordinances define sterilized milk as milk which has undergone such heat treatment as to render the product free from living bacteria. It may be sold only in sealed packs. In Denmark, UHT milk, if aseptically packaged in cartons, is treated as pasteurized milk for legal purposes. However, it is legally considered to be the same as in-bottle sterilized milk in the Netherlands. Generally, in Europe the term commercial sterilization implies that the product has been subjected to a processing temperature between 266 and 300 F with a holding time of at least 1 sec.

In the United States, the sterilization process is still an 'orphan' in the sense that there is no official (Federal or State) definition for sterilized milk products which are aseptically packaged in flexible paper containers. However, each milk operation that is processing sterilized products in the nation has adopted time-temperature combinations dependent on the nature of products processed and the equipment employed.

Future applications and opportunities

Sterilization and aseptic packaging, being relatively new processes which are of particular importance in terms of milk utilization potential, fill a void in dairy technology by solving the often encountered problem of very short shelflife of fluid milk products. Basically, any liquid milk product or fluid food can be sterilized and packaged aseptically, thus offering very significant product range flexibility.

To be successful in sterilized milk products operation it is essential to have properly qualified, experienced operators and technicians. Enough volume to keep the equipment busy for most of the day is also required. Any enterprise that has nationwide distribution or that packages numerous private brands should be successful. Such processing techniques, if applied to a central manufacturing plant, could also be very useful for a multifood organization which has a sale outlets throughout the country.

Sterilized dairy products may have a bright future in the U.S.A. even though adequate refrigeration facilities are available in this country. The need for production of "long life" milk products has long been felt by dairy processors and distributors. Increased education about sterilized products will no doubt bring about a great demand for them, but it will take time.

The dairy industry may reap benefits from application of sterilization and aseptic packaging if proper emphasis is given to: *(a)* quality of raw materials; *(b)* efficiency of processing and packaging equipment; *(c)* formulation, processing, and packaging of products; and *(d)* careful handling of finished products.

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INSECTICIDES USED ON DAIRY CATTLE AND IN DAIRY BARNS: TOXICITY TO MAN AND CATTLE, HAZARDS TO THE CONSUMER AND THE ENVIRONMENT[1]

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ABSTRACT

Insecticides currently in use for pest control on dairy cattle offer little hazard of toxicity to the cow, dairyman, consumer, or the environment. Pesticides that cause significant residues in milk cannot be used on dairy cattle or in dairy barns. Since the cow rapidly excretes pesticides from her circulating blood into milk, it is evident that only pesticides that are metabolized rapidly, and are safe to mammals, may be used. This consideration rules out use of the persistent pesticides that are hazardous by magnification in the food chain. The insecticides we rely on, mainly rapidly degraded organophosphorus compounds, are discussed according to: method of use; toxicity to cattle, laboratory rats, birds, fish, wildlife; degradation time; tolerance in milk; and hazard to the consumer of dairy products. Only a small amount of these insecticides enters soil and water following proper use on dairy farms. Their persistence is short. Degradation products are explored, noting their very low hazard for toxic environmental contamination.

INTRODUCTION

I shall limit myself in this discussion to insecticides used on dairy cattle or in dairy barns. These are the pesticides of most concern to dairy sanitarians. It should be noted, however, that the hazard of pesticide residues in dairy products, and of environmental contamination, are more likely to result from pesticides used to protect growing crops of dairy cattle forages, grains, and pastures.

My discussion will be from the standpoint of the compounds used and the purpose of their use, mammalian toxicity considering both cow and man, and potential for environmental contamination. At the outset, let me say that insecticides currently in use for pest control on dairy cattle present little hazard of toxicity to the cattle, the dairyman, the consumer, or the environment. Pesticides that cause significant residues in milk cannot be used on dairy cattle or in dairy barns. Since the cow rapidly excretes pesticides from her circulating blood into milk, it is evident that only pesticides that are metabolized rapidly may be used. This consideration rules out use of the persistent pesticides that are hazardous by magnification in the food chain. Thus DDT and other persistent chlorinated hydrocarbons have not been used for dairy cattle pest control for many years. We rely mainly on rapidly metabolized or degraded organophosphorus compounds such as crotoxyphos (Ciodrin), dichlorvos (Vapona), naled (Dibrom), trichlorfon (Neguvon), or botanicals such as pyrethrins and their very low toxicity synergists, or repellents. Somewhat more stable organophosphorus compounds [e.g., dimethoate (Cygon), fenthion (Baytex), ronnel (Korlan)] are used for residual fly control by treating barn walls, ceilings, etc. but only when no cows are in the barn. Even these compounds are rapidly degraded in manure, soil, and water so that hazard to the environment is minor.

A WORD ABOUT DDT

Despite the fact that chlorinated hydrocarbon insecticides in general are no longer used on or near dairy cattle, a word about DDT residues is in order. You may ask why we have a tolerance for DDT in milk — 0.05 ppm DDT + DDD + DDE in milk and 1.25 ppm in manufactured dairy products — when practically all use of DDT has been banned? The reason is that enough DDT, DDD, and DDE remain in soil, water, and air so that milk, and other food products, cannot be produced without a small residue of DDT or its metabolites. The amount allowed in milk and other food products is innocuous. It is far below a toxicological maximum "no effect" level, and far below levels fed in total diet to man in long term experiments, or rats in total life span trials, with no discernible ill effects whatsoever *(21)*. Also, I might add that there no longer is any purpose in using chlorinated hydrocarbons for house fly control in dairy barns, because our flies are highly resistant to these insecticides, and have been so for over 20 years.

The complexity of the environmental contamination problem is well illustrated by the PCB-DDT story. Research by gas chromatographic analysis for DDT delved into most conceivable environmental situations to study distribution and food chain effects. As a result, DDT was blamed for decreases in bird and fish populations, and shown to have circumworld distribution, even in antarctic penguins and arctic tundra. Some of the research was good,

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but some employed poor analytical procedures that confused polychlorinated biphenyls (PCBs) with DDT and DDT degradation products *(46)*. Then it was found that PCBs at very low levels had drastic effects on bird reproduction *(41)*. Some, but not all, of the onus was thereby removed from DDT and correctly placed on PCBs.

But the story has not ended there. Very recently it has been shown by Moilanen and Crosby *(33)* that intense ultraviolet radiation, of the same waveleigth as in sunlight, photoconverts DDT vapor through DDE into PCBs. Some of the unexplained disappearance of DDT degradation products from the world system might be explained in this manner, as might the odd worldwide distribution of PBCs coincident with DDT and degradation products. The main point for us is that the onus for deleterious effects of DDT on wildlife cannot be shifted entirely to the PCBs that we have used, because the PCBs in the environment may have originated partially from DDT pesticide application. And, when we largely absolve residues and metabolites of organophosphorus compounds of deleterious environmental effects, because they are not persistent, we may be oversimplifying.

Organophosphorus Compounds and Cholinesterase

All of the organophosphorus insecticides now in use poison insects or mammals by inhibiting the enzyme cholinesterase. This enzyme is vital to continued successful transmission of nerve impulses. So long as the insecticide, or those products of it which can inhibit cholinesterase, are present at the site of action, poisoning occurs. Cholinesterase is regenerated by the body, so that after the insecticide or its cholinesterase inhibiting metabolites are removed, return to normalcy can occur. Organophosphorus insecticides are not stored in the body for long periods as are chlorinated hydrocarbons. They are not cumulative poisons and are not passed on any distance through the food chain. Thus their action is short-lived so that they are not persistent environmental contaminants.

One hazard most important to pesticide applicators is related to rate of regeneration of cholinesterase. If a sprayman is exposed to a large but sublethal single dose of one of the more toxic organophosphorus compounds, he may have great depression of cholinesterase without experiencing very serious illness. In this condition, a second exposure to a similar dose could be fatal. In other words, although the insecticide is not cumulative, its effect over a period of several days may be. This effect has no bearing on low-level residues in food, which cause no cholinesterase depression.

Toxicity and Tolerance Table

Table 1 presents the various types of insect control used on dairy farms, the insecticides involved, their toxicities to laboratory rats and to cattle, rate of degradation, and tolerance in milk. These control methods plus precautions in using the pesticides and their tolerances are presented in detail by Matthysse *(31)*. In New York State, of all the insecticides used on or around cattle, only dichlorvos, fenthion and famphur in highly concentrated solutions are considered toxic enough to require a state permit to purchase them.

Fly Control by Residual Deposits (Table 1, Section I)

A mainstay of fly control on dairy farms has been residual deposits of insecticides on walls, ceilings, posts, partitions, etc. Flies walk on treated surfaces and pick up a lethal dose. For such a treatment to be practical, the insecticide must be stable and of low volatility so that a single application will continue to kill flies for several weeks. The amounts used are relatively large, commonly 1% insecticide in a spray applied at 1 gal to 300 to 1000 ft^2 of surface. Thus the barns for a 100 cow herd might have 50 gal of spray applied, containing 4 lb. actual insecticide. The several sprays for fly control through the fly season could result in as much as 8 to 20 lb. insecticide applied in 1 year. Fly resistance to these insecticides increases over a period of years, result-in more frequent application. There is a severe practical limit, however, Eventually fly resistance increases until the insecticide no longer provides control, as happened with chlorinated hydrocarbons and diazinon, malathion, and others.

For an understanding of possible hazard to the environment, we must put these amounts of insecticide in perspective. Suppose 20 lb. of insecticide was applied in a barn in 1 year on a medium-sized dairy farm, say 100 cows. If it all washed down into the manure, this could result in a maximum of about 0.2 lb. insecticide per acre on soil of some fields of the farm. My reasoning assumes that most of the insecticide left the barn via the manure, and that manure from 1 cow was spread on about 1 acre of farm land. This contrasts strongly with intensive pesticide use such as orchards where as much as 50 lb. of pesticide may be applied per acre annually.

Actually only a fraction of this 0.2 lb. per acre on a dairy farm would reach soil or water as the insecticides now in use are rapidly degraded in manure, soil, or animals. To date, there is no evidence of significant accumulation of these insecticides in the

TABLE 1. INSECTICIDES COMMONLY USED ON DAIRY CATTLE OR IN DAIRY BARNS

Insecticide	LD_{50} rats oral mg/kg[1]	Toxicity[2], to cattle; mg/kg oral, % spray: Older cattle	Young calves	Kind of compound	Degradation time	Ppm tolerance in milk
I. Used for spraying barns (residual fly control).						
Dimethoate (Cygon)	215	1% spray not toxic; 15 mg/kg toxic	1% spray not toxic; 50 mg/kg toxic	Organophosphorus	Moderate	0.002
Fenthion (Baytex)	215-245	25 mg/kg toxic	0.25% spray not lethal but cholinestrase reduced	Organophosphorus	Moderate	0.01
Ronnel (Korlan)	1250-2630	100 mg/kg toxic 2.5% spray safe	100 mg/kg toxic 2.5% spray no effect	Organophosphorus	Moderate	1.25 (fat)
Rabon	4000-5000	100 mk/kg not toxic; 0.5% spray not toxic	100 mg/kg toxic 2% spray toxic	Vinyl organophosphorus	Rapid?	0.5 (fat)
II. Used for spraying lactating cattle or into air in occupied barns (flies or lice and chorioptic mange mites)						
Pyrethrins (Pyrethrum)	820-1870	Safe because low dose used	Safe because low dose used	Botanical	Rapid	0.5 (fat)
Dichlorvos (Vapona)	56-80	25 mg/kg toxic 0.375% spray toxic	10 mg/kg toxic 0.5% spray toxic	Vinyl organophosphorus	Rapid	0.02
Crothoxyphos (Ciodrin)	125	>2% spray toxic 0.144% to 0.3% spray Brahma toxic	<2% spray toxic 0.5% spray safe	Vinyl organophosphorus	Rapid	0.02
Naled (Dibrom)	250	?	1% spray toxic	Organophosphorus (yields vinyl)	Rapid	0.05 (int.)
Synergists and repellents (piperonyl butoxide, MGK 264, MGK 326)	2800-7500	Relatively safe	Relatively safe	Various	Rapid	piperonyl butox .0.25 (fat) MGK 264 0.01 (int.) MGK 326 0.004
III. Used in milk rooms (fly control)						
Pyrethrins plus synergists and dichlorvos No-Pest Strips. See II.						
IV. Used in dust bags or backrubbers outside the milking barn, for lactating dairy cattle (flies and lice)						
Ronnel, Rabon, dichlorvos, crotoxyphos. See I, II.						
Coumaphos (Co-Ral)	13-230[3]	25-50 mg/kg toxic; 0.25% spray not toxic; 150 mg/kg pour-on toxic	0.25% spray toxic	Organophosphorus	Moderate	0.05 (fat)

V. Used on non-lactatnig dairy cattle, calves, heifers, dry cows (flies, lice, or cattle grubsQ.

Fenthion (Tiguvon), ronnel (Trolene), pyrethrins, dichlorvos, crotoxyphos, synergists and repellents, comaphos. See I, II, IV.

Methoxychlor	5000	Spray not lethal	500 mg/kg toxic but spray not lethal	Chlorinated hydrocarbon	Moderate	0.05 (int.)
Malathion	1000-1375	100 mg/kg toxic 2% spray max. safe	20 mg/kg toxic 1% spray toxic	0.3% lethal	Moderate	0.1 (fat)
Crufomate (Ruelene)	460-635	100 mg/kg toxic 2% spray not toxic	50 mg/kg toxic 2% spray toxic	Organophosphorus	Moderate	
Trichlorfon (Neguvon)	560-630	75 mg/kg toxic 2% spray not toxic	10 mg/kg toxic 1% spray safe	Organophosphorus (yields vinyl)	Rapid	0.01
Famphur (Warbex, Fanix)	35-62	50 mg/kg toxic	10 mg/kg max. safe	Organophosphorus	Moderate	

VI. Used in fly-baits in barns (house fly)

Dichlorvos, naled, malathion, trichlorfon (Dipterex). See II, V.

Diazinon	76-108	over 25 mg/kg toxic; 0.25% spray not toxic	2.5 mg/kg toxic 0.1% spray toxic	Organophosphorus	Moderate	

VII. Never to be used, presented for comparison only

DDT	113-118	500-2000 mg/kg min. toxic dose; 22g/steer weekly 3 years no effect	250 mg/kg min. toxic dose, 8% spray not toxic	Chlorinated hydrocarbon	*Slow*	0.05
Lindane	89-91	25 mg/kg lethal 0.3% lethal	5 mg/kg lethal .05% spray can be lethal	Chlorinated hydrocarbon	Slow?	
Parathion	4-13	50 mg/kg not toxic	0.5 mg/kg toxic 0.01% spray toxic	Organophosphorus	Moderate	

1. Toxicity *Classes* based on rat oral LC^{50} are: 1. Highly toxic 1-50, 2. Moderately toxic 50-500, 3. Slightly toxic 500-5000, 4. Relatively non-toxic over 5000. Dermal toxicity 4 times higher in each class. Toxicity in Classes 3 and 4 are considered low hazard, acceptable for home owner use with no protective equipment.

2. "Toxic" indicates symptoms of poisoning produced, but not necessarily death. "Lethal" means that the dose in question will cause death of some treated cattle.

3. Coumaphos—Sex difference in toxicity to rats; male LC^{50} 56-230 mg/kg, female 13-30.

Toxicity to cattle derived from Radeleff (44), from unpublished data of J. S. Palmer of the U.S. Department of Agriculture at Kerrville, Texas, and from manufacturers' reports. The rat toxicity is from a compilation by J. E. Dewey, Pesticide Coordinator, Entomology Department, Cornell University, Ithaca, N.Y.

environment, in the food chain, or in agricultural produce. And their degradation products are of a very low order of toxicity, and short-lived in the environment.

The mammalian toxicities of insecticides used for residual fly control fall into two groups. Dimethoate and fenthion are moderately toxic, requiring greater caution in use than ronnel or Rabon which are only slightly toxic.

As to contamination of dairy products – all insecticides that have been approved by the federal Office of Pesticide Programs, Registration Division, Environmental Protection Agency for residual fly control have been thoroughly tested for residues in milk and found to produce either no residues, or negligible residues of no toxicological significance and well below tolerances in dairy products. Poulsen *(43)* states that use of organophosphorus insecticides in barns does not, as a rule, cause residues in tissues and milk of animals, provided the feeding and watering troughs and the feed and water are not contaminated with the insecticide and the insecticide is not applied to the animals. He states that cows should be removed from buildings and the milk processing rooms should not be sprayed. All precautions should be taken to prevent direct contamination of milk and utensils.

SPRAYING DAIRY COWS OR MISTING IN THE BARN (TABLE 1, SECTION II)

Insecticides used for direct spraying of dairy cows, or misting into the air of occupied barns, are all rapidly degradable compounds that offer no significant hazard of environmental contamination or toxic residues in dairy products. Pyrethrum, synergists (compounds that increase the effectiveness of pyrethrum) and repellents are of a very low order of mammalian toxicity. Metcalf *(34)* notes that pyrethrins are generally regarded as among the very safest of insecticides and that this safety is partly due to an inherent lack of toxicity and partly due to the instability of the compounds in light and air so that toxic residues quickly disappear. Pyrethrins were fed to rats for 2 years at 1000 ppm without causing any tissue damage. Also, the low concentration and small volume (1 to 2 fl oz per cow maximum) used in spraying dairy cows insure against hazard of toxicity or significant contamination. Pyrethrum was the insecticide of choice for oil-base fly sprays because its rapid knock-down of flies quickly cleared barns of these pests. In the past 10 years, pyrethrum has been partially replaced by vinyl or vinyl-producing organophosphorus insecticides. Dichlorvos (Vapona), naled (Dibrom), and crotoxyphos (Ciodrin) give quick kill of flies, and undergo very rapid degradation to innocuous products. These compounds are in the moderately toxic class, warranting greater caution in their use.

The quantity of these fly spray ingredients used on dairy farms is small. For example, 1% crotoxyphos spray is commonly used at 1 to 2 fl oz per cow. About 0.1 lb. crotoxyphos would be used for one spraying of a 100-cow dairy herd. Even with daily use during a 5-month fly season (far more than dairy farmers use in practice), the total poundage used (15 lb.) is approximately the same as calculated previously for residual fly control. And these fly spray ingredients are of less contamination significance than the insecticides used for residual fly control.

A slight shadow on safety of synergists has been cast by Falk and Kotin *(8)* who noted their possible general action in blocking or delaying the metabolic degradation of hormones, pharmaceutical agents, and toxicants including carcinogens. However, at rates used on cattle, residues of synergists in milk and dairy products are either non-existent or so low as to preclude probability of such side effects.

The vinyl organophosphorus compounds dichlorvos and crotoxyphos, and naled which is activated to dichlorvos, produce no detectable residues in milk when used properly. Dichlorvos is not stored in the body nor excreted in the milk to any appreciable extent even when administered in doses that produce severe poisoning *(10)*. Cows fed diets containing up to 2000 ppm dichlorvos for 8 days did not excrete dichlorvos or toxic metabolites in the milk (Shell Development Co., unpublished report).

INSECTICIDES IN THE MILK ROOM (TABLE 1, SECTION III)

Insecticide use in milk rooms has been severely restricted, to prevent all possibility of direct milk contamination. The relatively safe natural botanical, pyrethrum, is used along with synergists. Recently, dichlorvos Pest-Strips (20% dichlorvos in a polymer) have been approved for residual fumigation. It is conceivable that milk could absorb dichlorvos from air containing dichlorvos in vapor phase. However, no significant residues result in milk because of rapid hydrolysis. Gillett et al. *(16, 17)* conclude "experiments to date and a critical review of literature and information available on dichlorvos resin strips do not reveal any substantial hazardous effect on human beings."

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FLY BAITS (TABLE 1, SECTION VI)

A number of the insecticides previously discussed are also used in sugar-base fly baits, commonly call-

ed "scatter-baits". Fly baits are more universally used by dairymen than any other fly control device. The organophosphorus insecticides used in baits are all rapidly degradable vinyl or vinyl producing compounds, or have very low mammalian toxicity. An exception may be diazinon. In general, baits are eventually swept into manure where degradation of these compounds is rapid. We think that baits should not be used in the milk room where direct contamination of milk is always a possibility. A saucer of bait and a cat on top of a bulk tank is simply an inexcusable practice.

Dust Bags and Backrubbers (Table 1, Section IV)

A slightly more expanded list of organphosphorus insecticides is accepted for use in dust bags and backrubbers as self-treatment devices for milking cows. Absorption through the skin from these low insecticide concentration dusts, and from oil solutions used in very small volumes, is rather limited. Residue studies have proven either no excretion into milk, or residues well below tolerance. Hazard to the environment is most unlikely because of the small amount of low-toxicity degradable insecticide used in these devices.

Insecticides for Use Only on Non-lactating Cattle (Table 1, Section V)

Despite the rapid mammalian metabolism of organophosphorus compounds, many of them will cause residues, albeit extremely small, in milk subsequent to dermal or oral application to lactating dairy cows. Compounds which should be used only on non-lactating dairy cattle (calves, heifers, dry cows, bulls) and beef cattle for control of flies, lice, and cattle grubs include famphur (Warbex, Famix), trichlorfon (Neguvon), crufomate (Ruelene), malathion, fenthion (Tiguvon, Baytex), and certain formulations of coumaphos (Co-Ral) and ronnel (Korlan). The unusually rapidly metabolized chlorinated hydrocarbon methoxychlor is also used in this manner against lice and flies. These insecticides are "slightly toxic" or "moderately toxic" except for famphur and coumaphos which border on the highly toxic category. These insecticides do accumulate for a short time in the tissues of cattle, and are excreted into milk. To prevent milk contamination, they must not be used close to freshening. This period varies from 3 days for crufomate (Ruelene) to 28 days for fenthion (Tiguvon).

MacDougall *(30)* reviewed milk residue data for various applications of coumaphos, fenthion, ronnel, trichlorfon, crufomate, and famphur. Of the 11 treatments considered, 3 gave residues of $<$ .01 ppm in milk immediately after treatment, and 2 more were $<$ .01 ppm 1 day after treatment. By 3 days, 7 of 11 treatments gave residues of $<$ .01 ppm, and by 7 days only 1 treatment gave a residue $>$ .01 ppm. Note that these were strong concentration treatments (.25% to 12.5% insecticide in sprays, pour-ons, and backrubbers), mainly of the type used against cattle grubs and *not* recommended for use on lactating dairy cows. Recommended treatment for lactating dairy cows produce negligible or extremely low residues of no toxicological importance.

Of course, all insecticides accepted for use on lactating cows may also be used on non-lactating cattle, with no period required between treatment and freshening. However, none of the insecticide concentrations allowed on lactating cows are effective against cattle grubs. Rotenone, a rapidly degraded botanical insecticide of low mammalian toxicity, was used for many years on milking cattle for grub control. Recently, all livestock uses of rotenone were cancelled because of indequate data on metabolism, and lack of residue tolerances. We have no accepted insecticide for use against cattle grubs on lactating dairy cattle.

Treating Manure to Kill Fly Maggots

Dimethoate (Cygon), Rabon, and ronnel (Korlan) are labelled for application to manure to kill fly maggots. We do not recommend such larvicide treatment as a general practice because it is a most efficient way to produce insecticide resistance in house flies, and because of destruction of parasites and predators of house fly eggs and larvae. Larviciding requires a large amount of insecticide applied frequently, whenever fresh manure is added to a pile, or pack builds up under penned cattle. A common dose is 1 lb. actual insecticide per 1250 ft.^{2} of manure surface. Weekly applications during a 4-month fly season requires 17 lb. of actual insecticide on one 25 by 50 ft. pen of cattle. Even though environmental contamination is greater than any of the previously discussed uses, it is still unlikely because of rapid degradation of these organophosphorus compounds. However, killing arthropod fauna of manure spread on fields or in patties on pasture might delay return of the nutrients to the soil and decrease pasture and crop productivity. For the same reason, use of insecticides as feed additives for fecal fly control may be a poor practice.

Insecticides That Should Never Be Used On The Dairy Farm (Table 1, Section VII)

DDT and lindane are two chlorinated hydrocarbons that should never be used on cattle because of

illegal residues resulting in milk and meat. Note that DDT is not very toxic to cattle and that lindane is much more toxic. Parathion is also presented for comparison in this table. Its extreme toxicity to rats and calves is evident, but it appears from these data to be relatively safe on cows. Do not be mislead. As a spray it is highly toxic. Because of breakdown in the rumen by microorganisms, it is not so extremely dangerous by *oral* dose to cattle.

ENVIRONMENTAL CONTAMINATION HAZARD BY INSECTICIDES AND THEIR METABOLITES

Insecticides applied in or around dairy barns may be toxic hazards to man or the environment by departing the site in milk or meat, in manure and thereby the soil, in effluent water, or in air. Metabolites and degradation products must be included in evaluating this hazard.

The organophosphorus compounds in general are converted rapidly to water soluble ionizable compounds that are not cholinesterase inhibitors and are relatively non-toxic. Hydrolysis of the phosphorus ester bonds is usual, producing methyl or ethyl mono- or di-phosphates or thiophosphates from the phosphorus containing moiety of the parent compound *(9-12, 14, 15, 22, 30, 36-38)*. Oxidative metbolism also occurs with these compounds *(6, 29)*, finally producing water soluble metabolites of little toxicological significance. Further degradation produces phosphoric acid with much of the phosphorus entering, indistinguishably, the phosphorus pool of the body or of the environment. None of these degradation products offer significant toxicity hazard.

The remainder of the organophosphorus insecticide molecule varies greatly. Nevertheless, in general, water soluble, ionizable, relatively innocuous degradation compounds are produced. Most rapid degradation occurs with the vinyl compound dichlorvos, and with trichlorfon and naled which rearrange to dichlorvos, with loss of HCl and HBr, respectively. The non-phosphorus moiety degrades through dichloroacetaldehyde, dichloroacetic acid, and dichloroethanol. Glyoxal is also produced by further reaction. As with phosphorus, much of the carbon ends up as normal body components of intermediary metabolism (Shell Development Co. unpublished report). Toxicity of the dichloro metabolites is low. Dichloroacetaldehyde and dichorobromoacetaldehyde given orally to rats at 200 and 120 mg/kg/day respectively, showed no evidence of toxicity (Chevron Chemical Co. unpublished reports).

The vinyl phosphates Rabon and crotoxyphos are somewhat more stable, but degradation in animals and in the environment is adequately rapid. Crotoxyphos is very rapidly absorbed and excreted by animals (44). Maximum blood concentration of crotoxyphos in sheep occurred 6 h post oral dosing, and declined to 1/10 the concentration at 48 h. Almost 80% of the dose was eliminated as urinary metabolites within 48 h. Dimethyl phosphoric acid comprised 90% of the detectable phosphorus excretion. Rabon fed to rats at 125 ppm or less daily for 2 years caused no adverse effect (Shell Chemical Co., unpublished report). Benyon and Wright *(4)* determined degradation products of Rabon in soil and concluded that "the breakdown products are of low toxicity to rats and apart from the α isomer, none . . . inhibits cholinesterase."

The following are examples of degradations of the non-phosphorus moieties of several of the more complex organophosphorus compounds. The carboethoxy moieties of malathion hydrolyze to mono- and di-carboxylic acids *(37)*. The N-methyl-carbamoyl methyl moiety of dimethoate is metabolized to the carboxyl derivative *(14, 37)*. These are water soluble, rapidly excreted, ionizable compounds that are not cholinesterase inhibitors and are relatively innocuous. With coumaphos, the chloromethyl coumarin radical is metabolized to chlorferron, which is of a low order of toxicity *(12)*. The trichlorophenyl radical of ronnel is metabolized and excreted as trichlorophenol *(12)*. Trichlorphenol fed to rats at 1000 ppm or less daily for 98 days caused no adverse effects. Ronnel itself fed to rats at 15 mg/kg or less per day for up to 2 years caused no adverse effects. The hydrolytic metabolite of the 4-tert-butyl-2-chloro-phenyl radical of crufomate, the corresponding phenol, fed to rats at 1000 ppm or less daily for 2 years caused no adverse effects *(12)*.

PERSISTENCE IN SOIL

The organophosphorus insecticides used in or around dairy barns may enter soil through the spreading of manure. They are classed as nonpersistent in the U.S. Department of Health, Education and Welfare Secretary's Commission Report *(2)*. Half lives in soil for several pertinent pesticides are: malathion—8 days; dimethoate—2½ to 4 days; diazinon—17 to 18 days; Rabon—5 to 11 days *(4)*; crotoxyphos— 1 h to 3 days; (5, 27, Shell Development Co. unpublished report). However, soil type markedly affects persistence of insecticides.

Harris *(19)* divided insecticides into 3 groups according to persistence in soil, as assessed by biological activity. Group 1, chlorinated hydrocarbons such as DDT, persisted more than 48 weeks without significant decrease in activity. Compounds in Group

2 are not pertinent to this discussion. Group 3 which included diazinon, disappeared within 2 to 4 weeks. This reasearch was done in the laboratory. Harris and Hitchon *(20)* confirmed <4 weeks persistence for diazinon in the laboratory and added a persistence period of slightly over 24 weeks for dimethoate. Read *(45)* however, working in the field, found rapid loss of diazinon during the first month, but persistence at low levels for up to 3 months. Kearney et al. *(25)*, in their review of persistence of residues in soil, concluded that phosphate insecticides are short-lived in soil. They state that diazinon is the most persistent, remaining for only 3 months, and show malathion as persistent for <1 week. In contrast, some chlorinated hydrocarbon insecticides persist for many years in soil. Accumulation of organophosphorus compounds in soil to a significent degree for toxic pollution is most unlikely. The degradation products, as noted previously, do not present a significant hazard. Duff and Menzer *(7)*, concerning dimethoate degradation products in soil, stated: "This material does not contain neutral phosphorus esters, and it is, therefore probably non-toxic."

Contamination of Water

Insecticides in general are unlikely to contaminate ground water because they bind tightly to soil particles. Lichtenstein et al. *(28)* analyzed percolation and found little or no pesticide, but noted that runoff transport of soil particles will carry pesticides into streams. Again, most dairy insecticides break down rapidly in water, for example the half life of dichlorvos in water is 8 h and dibrom is destructively hydrolyzed in about 2 days *(35)*. Trichlorfon half life for conversion to dichlorvos is 6 h. in water *(15)*. However, we must guard against direct contamination of watery effluent from barns. Insecticides used for residual treatments for fly control are longer-lived in water. Nevertheless, the degradation products likely to be spread on soil and washed into streams are not considered hazardous toxicants to fish or wildlife.

Hazard to Fish and Wildlife

Considering hazard of toxic effects on fish and wildlife, this does not always parallel the toxicity to rats or cows that I have stated previously. Fenthion is one of the more toxic compounds to birds. Heath et al. *(23)* give LC_{50}'s for fenthion to 4 bird species (2-3 weeks old) as 20-231 ppm in feed, fed 5 days. The following are approximate average dose ratios compared to fenthion for LC_{50}'s to several wild bird species (the higher the figure the less toxic the insecticide): diazinon 1.3, parathion 1.6, coumaphos 2.5, dimethoate 4, DDT 6, trichlorfon 10, naled 16, malathion 27 or more, and methoxychlor and dichlorvos over 36 *(23)*. It should be noted that susceptibility differs among bird species. Regardless, trichlorfon, naled, methoxychlor, dichlorvos, and malathion are relatively safe in food of birds but care should be exercised against exposure of poultry or wild birds to fenthion, diazinon, coumaphos, or dimethoate. Care should also be exercised with crufomate and famphur. Also relatively safe are Rabon, crotoxyphos, ronnel, pyrethrins, synergists, and repellents. Rabon up to 800 ppm in the diet of quail for two weeks caused no observable toxicity. Acute oral LD_{50} of crotoxyphos to mallard ducks is 790 mg/kg (Shell Development Co. unpublished report). The order of decreasing *dermal* toxicity to chickens (by dipping the birds completely) according to Foulk and Matthysse *(13)* is: diazinon (lethal at < .035%), fenthion, dichlorvos, naled, coumaphos, dimethoate, crotoxyphos, malathion, ronnel (lethal at 5% or more). Nevertheless, coumaphos is labelled for direct use on chickens at proper dosages.

I have shown previously that but a small amount of insecticide,much < 1 lb. per acre, is likely to be put on land by manure from barns in which pesticide is applied. Anon *(1)* states that 1 lb. of diazinon per acre is not hazardous to pheasants—and diazinon is one of our most toxic insecticides to birds.

Fish are very susceptible to most of the insecticides we are discussing. Willford *(48)*, using six species of fish, found 24-h LC_{50} (ppm in water) that averaged: ronnel 1.2, coumaphos 3.7, fenthion 7.4, crufomate 35.1, trichlorfon 53.3. Naled is toxic to fish, crustaceans, and shellfish; LC_{50} values vary from .08 ppm to 4 ppm (Chevron Chemical Co. unpublished report). LC_{50} for dichlorvos is < 10 ppm. It is evident that careless use of ronnel, coumaphos, naled, or fenthion, resulting in direct contamination of water with spray or immediate barn effluent, could be dangerous to fish. Trichlorfon and crufomate are less hazardous. Hair and Howell *(18)* found no deleterious effects by Rabon on fish from direct application of 1 lb. per acre.

Pimentel *(42)* reviewed toxicities to fish showing considerable variation among species and from the data by Willford *(48)*. We must add the following to the list of insecticides that are very hazardous to fish: pyrethrins and pyrethroids, rotenone, diazinon, malathion, methoxychlor, naled, and dichlorvos. Dimethoate is less toxic to fish but by degree only. None of these insecticides must be allowed to contaminate streams, ponds, or lakes.

Contamination of waters would also be deterimental to arthropod stream fauna that is vital as food for fish. Pimentel's *(42)* review showed that all the

pesticides we use in and around dairies are very toxic to these beneficial insects and crustaceans. It is obvious that there is need for caution against direct contamination of streams and effluent water by barn spray run-off, spray or dust drift during outdoor treatment of cattle, or contamination from draining or cleaning sprayers. The toxicity of pesticides to non-target species has been summarized by Pimentel *(42)*.

CONCLUSION

In conclusion, insecticides currently in use for pest control on the dairy farm will not produce hazardous or illegal residues in milk or manufactured dairy products, when used as directed. The trend is towards pesticides of low toxicity to man and animals, and those which undergo rapid methabolism or degraduation to products offering no hazard to man or to the environment. However, these conditions are true only if the farmer or commercial pesticide applicator adheres strictly to the label directions and cautions. Overdose, wrongly timed application, or violation of cautions can produce excessive residues or even hazard to the cattle or the applicator. The new Federal Environmental Pesticide Control Act, now in force, makes it illegal to apply pesticides other than as directed on the label.

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MICROBIOLOGY OF EGGS[1]

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ABSTRACT

It is generally agreed that milk and eggs are nature's most perfect foods. Milk is extremely perishable while eggs in the shell are not. The reason that eggs are not as perishable as most foods is that they possess protective devices including the cuticle, shell, shell membranes, lysozyme, conalbumin, avidin, pH of the albumen, etc. Having these protective devices is nature's way of allowing reproduction of birds. Chicken eggs, for example, take 21 days to hatch under ideal conditions for growth of microorganisms. Without the protection, reproduction could not take place. Once eggs are broken out of the shell and mixed, they are as perishable as milk. Pathogenic bacteria of concern to humans found in eggs (particularly liquid eggs) are *Salmonella, Staphylococcus aureus,* and *Clostridium perfringens.* These pathogens are not common in shell eggs but can be found in liquid eggs primarily because of reinfection by humans.

It is generally agreed that milk and eggs are nature's most perfect foods. Milk, on one hand, is extremely perishable while eggs in the shell are not. The reason eggs are not as perishable as most nutritious foods is that they possess several protective devices which include the cuticle, shell, shell membranes, lysozyme, conalbumin, avidin, pH of the albumen, and so forth.

I will limit my discussion to eggs and bacteria because bacteria are more bothersome to eggs by far than other microbes. Molds will affect eggs on occasion but this is not common today. What I intend to do in this paper is to describe how eggs are infected with bacteria and hope that you have a better appreciation of bacteria and eggs when I finish.

When a hen lays an egg, she doesn't intend for us to eat it. This is not what she has in mind at all. She intends that the egg is for reproduction. For a chicken egg to hatch, it must remain under ideal conditions for microbial growth for 21 days. If an egg didn't have protective devices against bacteria or other microbes, there would be no possible way that reproduction could take place. Nature does a wonderful job in protecting the egg. Although the egg seems simple, it is very complicated and even though scientists have studied it for many years, there are many factors about eggs still not known, particularly in the area of protective mechanisms.

When you stop to think about it, the egg is remarkable. You can put eggs out on the kitchen table and two months later, they are still likely to be edible. One cannot do this with other foods as high in nutrition and water as the egg. The same nutrition that we obtain from the egg as humans is also beneficial to bacteria.

THE CUTICLE

To understand how bacteria get into eggs, it is important that one knows the parts since the egg is extremely complicated. Bacteria try to go to the yolk of the egg, which is really where the nutrients are and thus it is the target for the organisms. If bacteria work from the outside in, the first barrier involved is the cuticle which is largely protein. If we were to dip an egg into a weak hydrochloric acid solution, the cuticle would swell up and one could gather as much as a full teaspoon. The purpose of the cuticle is to protect the egg when it is first layed. Eggs are layed in places where there are usually many bacteria, and they need protection at this time. When an egg is first layed, it is at 107 F and the temperature drops to ambient in a short time. This drop in temperature creates a tremendous force inward, enough to suck in bacteria. With the cuticle, however, this does not happen. Thus it is a way of protecting the egg when it is first layed.

We know the cuticle gives protection for at least 100 h because we have challenged eggs at time intervals for 100 h and the cuticle still gives protection. How long it protects beyond 100 h is not known. We feel that the cuticle eventually dries out and cracks and then bacteria can enter the pores of the shell. We have taken eggs from the hen before the cuticle is put on, and we find that these eggs do not resist the challenge nearly as long as eggs with cuticle. Hence, the cuticle is extremely important.

THE SHELL

The next layer that bacteria come to is the shell. The shell has thousands of pores. We have made many studies on pores of the egg shell because we are interested in learning where bacteria enter. We find most of the pores are in the equatorial region of the egg and in the big end. Our research has shown that most bacteria enter the big end of the egg in the area of the air cell. The shell of an egg is not a particularly good barrier; in fact without

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the cuticle the egg will become infected much sooner. The shell does give some protection but it's not extensive.

SHELL MEMBRANES

The next layer that bacteria encounter is the outer shell membrane. Perhaps you never knew that eggs have membranes because you haven't studied or observed them. The outer shell membrane is five times as thick as the inner shell membrane, but it does not give nearly as much protection as the inner shell membrane. The outer shell membrane does give some protection, but I will emphasize the inner shell membrane because it is much better.

The air cell is formed between the two membranes. Usually, it is formed at the big end because this is where the two membranes separate the easiest. There is no air cell when the egg is layed but during contraction, it is formed. As the egg dries out, the air cell gets larger. This does influence quality because air cell size is a quality factor. As the air cell gets larger, it has some influence on the sucking in of bacteria.

The inner shell membrane is much thinner and weighs less than the outer shell membrane but it effectively protects against bacteria. We have done considerable work at Cornell University on the inner shell membrane and in many respects, it is still a mystery to us as far as bacterial penetration is concerned. The inner shell membrane is very high in lysozyme. There is also some lysozyme in the outer shell membrane but not nearly as much as in the inner shell membrane. Lysozyme is an anti-bacterial agent and is effective against gram-positive bacteria, but not particularly against most gram-negatives. This means when a bacteriologist examines an egg, he may find gram-negatives but not many. This is a major reason why eggs don't spoil as fast as other foods similar in nutrition. Many of the bacteria are destroyed by the lysozyme in the inner shell membrane. In addition to the lysozyme, no pores can be found in the inner shell membrane. We have not been able to find pores even with the electron microscope. This is a mystery because without pores or holes, how do bacteria get through? Reasoning would say that the only way bacteria could possibly go through would be to create an enzyme system. We thought this was an easy solution but we have used every proteolytic and lipolytic enzyme known and we have not been able to influence the inner shell membrane. Our theory at this point is that the inner shell membrane is made up of a matrix of many fibers and these fibers are so interwoven that there are no holes that go straight through the membrane. A bacterium, however, if it is active, can wiggle its way among the fibers until it gets through. We think we have proven this to a certain extent because if we cause bacteria to be inactive by using sulfa drugs, these bacteria will not penetrate. If the bacteria do get through the inner shell membrane, normally one cannot find them for sometime in the albumen. That was a mystery to us for many years but we finally discovered that they congregate on the inside of the inner shell membrane until they accumulate in numbers great enough to invade the albumen.

THE OUTER THIN WHITE

The next layer is the outer thin white. Although we haven't done a great deal of research on the outer thin white, we can compare it to the outer shell membrane as far as protection is concerned. There is some anti-bacterial activity due to pH since the pH is not ideal for bacterial growth, but beyond this we don't think there is much anti-bacterial activity.

THE THICK WHITE

The next part or layer is the thick white. Most people think that the thick white is continuous to the yolk but this is not true. It is just a very thin envelope that completely surrounds the yolk. If one looks at the top of the yolk, one will see the exact thickness of the thick albumen. The thick albumen contains lysozyme which again will destroy any gram-positive bacteria that may have penetrated to this point. We also find in the thick white a protein and a polypeptide that are important in resisting bacterial penetration. The protein is conalbumin which chelates iron. Bacteria need iron and if it is not available, they don't do well. In regions of our country where there is a great deal of iron in the water, bacteria may carry enough iron in their cells to supply their needs, and in those areas we have more trouble with egg spoilage. The polypeptide is avidin, which chelates biotin. Many bacteria need biotin and thus they don't do well.

INNER THIN WHITE

The next area bacteria find is the inner thin white. This is a very watery albumen and there is a large amount of it in every egg. As far as we know, the inner thin white doesn't have much anti-bacterial activity except the pH is not ideal for bacterial growth.

THE CHALAZIFEROUS LAYER

Before the bacteria get to the yolk, they have to penetrate the chalaziferous layer. This layer is very viscous albumen and has everything that the thick white has but in higher concentrations. The chalaziferous layer protects the yolk. The ends of the chalaziferous layer are milky white cords that are known as chalaza cords. These chalaza cords anchor the yolk in the center of the egg and they are important because they hold the yolk so it is protected by the thick white. If it wasn't for the chalaza cords, the yolk would float to the shell and, of course, eggs would decay much faster. Without chalaza cords, eggs wouldn't hatch and thus reproduction of the species would be inhibited.

VITELLINE MEMBRANE

The last layer and probably not a very important one as far as bacterial penetration is concerned is the vitelline membrane, which surrounds the yolk. The main purpose of this membrane is its impermeability to water, hydrogen sulfide and other elements going into the yolk and such elements as iron coming out of the yolk.

BACTERIA IN EGGS

As a general statement I can say that most any bacteria can get into an egg. We can find most bacteria but, as stated earlier, the gram-negatives predominate.

The pathogenic bacteria in eggs are probably of greatest interest and so I will devote the rest of this paper to *Salmonella, Staphylococcus aureus,* and *Clostridium perfringens.*

Salmonellae are of great importance and have received much publicity in conjunction with eggs. Much of this publicity goes back to the day when eggs were infected heavily with one species of salmonellae, known as *Salmonella pullorum.* Not too many years ago all chickens had Pullorum disease. The bacteria were found in the ovary and thus most eggs were infected. As far as we know, this is the only bacterium that can be transmitted through the egg and can be found in the egg when it is layed. Today, to find *Salmonella pullorum* would be quite a feat indeed. We haven't found salmonellae in eggs in several years. The egg industry, however, is still paying for its sins of many years ago.

Many species of *Salmonella* can be found on the shells of eggs because they are common in the intestinal tracts of chickens. Salmonellae are not particularly active in penetrating unless eggs are improperly handled. One of the problems in infection of eggs is the contraction. If there should be contaminated water on the shell when the egg is cooling off, then bacteria can be sucked through. It is always important to remember that if eggs are washed, cold eggs should be placed in warm water so the eggs are expanding rather than contracting. To do the opposite is very dangerous because bacteria can be sucked in.

I think I can say with accuracy that the incidence of salmonellae in shelled eggs is not very great. Many people worry about using cracked eggs but, again, remember that I said the shell gives little protection.

With liquid eggs, the problem of salmonellae is different. A few years ago, it was not legal to use a sanitizer-detergent on eggs that were to be broken out. Today industry people wash eggs with sanitizer-detergents and leave them on the shells, which maintains sanitized shells for a long time. In the days when the industry did wash eggs but without a sanitizer-detergent, there were salmonellae on eggs when they were broken. The egg albumen would run to the liquid product. At that time, salmonellae down over the shell and carry salmonellae with it inwere detected in some liquid eggs and this gave eggs a bad name. Today, most salmonellae infections in liquid eggs come from reinfection of the egg and, in most instances, from humans. Although liquid eggs are pasteurized they can be reinfected after pasteurization.

The next pathogen I would like to consider is *Staphylococcus aureus. S. aureus* can cause illness in humans in a short time. The incubation period is much less than that of salmonellae, although the two are confused from time to time. In eggs, the chance of *S. aureus* getting into a shell egg is very remote because it is a gram-positive organism. In egg products, however, such as liquid egg, there is a chance of the organism getting in, and again it comes mainly from humans since *S. aureus* is found in large numbers in sores and cuts on hands of employees.

The last pathogen of real importance in eggs is *Clostridium perfringens,* which is sometimes known as *Clostridium welchii.* It has only been of concern recently, and the concern really came from England where they have less refrigeration than in this country. Again, the chance of *C. perfringens* being found in shell eggs is very remote because it is a gram-positive organism. However, it can be found in liquid eggs because it is a widly distributed organism. We've found that *C. perfringens* can be found almost any place. Fortunately, only a few strains of the organism are pathogenic and even then the pathogenicity is not very high. It is my personal belief that many times when illness is diagnosed as salmonellosis, the real cause is the toxin from *C. perfringens.* We will

hear much more about *C. perfringens* in the future.

I could discuss the many bacteria that are found in eggs but those already mentioned are the ones of importance as far as illness to humans is concerned. Many of the gram-negatives can be found (sometimes in high numbers) and one of the primary causes of spoilage is *Pseudomonas*. One of the testing methods for bacterial infection in eggs is the ultra-violet light. For those *Pseudomonas* that fluoresce in ultra-violet light, the internal part of the egg will give a green color.

IN CONCLUSION

In closing, I have emphasized that shell eggs have many barriers against bacteria. Even with all these barriers, eggs can become infected because some people tend to grossly misuse shell eggs. The number of spoiled eggs that come into our laboratory today, however, is only a fraction of what it was 20 years ago.

I would like to emphasize, however, that with liquid eggs (we are moving into the liquid egg business very rapidly), the situation is entirely different. Once yolk is mixed with albumen, all of the anti-bacterial properties of the egg are removed. First of all, we don't have the cuticle, shell, and the shell membranes for protection because they are thrown away. Because of the blending of yolk with the white, we are losing any protection we might get from the albumen; thus, mixed whole egg is an ideal medium for growth of bacteria. Liquid eggs have a shelf life very similar to that of milk. If one starts with shell eggs with low numbers of bacteria, breaks them out under sanitary conditions, and pasteurizes them, one can get up to about 20 days of shelf life. On the other hand, if one starts with shell eggs that are very high in bacteria counts, even with pasteurization, one can only get 2 to 3 days shelf life.

EDUCATION AND INFORMATION PROGRAM ABOUT FOOD PROCESSING INDUSTRY BOWS AT NATIONAL EXPOSITION FOR FOOD PROCESSORS

An information and education program designed to emphasize the contributions made by the Food Processing Industry to the American life-style has been launched here during the industry's annual trade show and convention.

The program, sponsored by the Food Processing Machinery and Supplies Association (FPM&SA), was presented during the association's National Exposition for Food Processors (NEFP), the largest annual trade show in the industry. The NEFP and the concurrent National Canners Association convention attract thousands of representatives from every aspect of the Food Processing Industry whose annual production is valued at more than 10 billion dollars.

The major thrust of the program is a series of posters which depicts some of the benefits Americans enjoy as a result of processed and manufactured foods. FPM&SA also has assembled a digest of statistics which highlight the industry's technological advances and its role in the Nation's economy. Initially, the program is being directed at the thousands of employees who produce, prepare, process, and package the canned, frozen, dehydrated, baked, and otherwise manufactured food products. The posters are being supplied free of charge to food processors and their suppliers for display in about 4,000 operating plants and sales offices throughout the United States.

The osters also are suitable for use in print advertising, according to FPM&SA, which is urging industry firms to disseminate the material in their localities. "Recent inflationary trends in the economy have tended to overshadow the real contributions the Food Processing Industry has been making and will continue to make to American life-style," the association stated. "Our program simply seeks to reemphasize the positive impressions consumers have about processed foods, and to disseminate some little-known facts about the Industry."

FPM&SA choose the NEFP and Canners Convention as the vehicle for introducing the campaign since the displays and meetings at this annual four-day gathering reflect much of the technology and innovations which have allowed the Food Processing Industry to contribute significantly to the American life-style and economy.

OPPORTUNITIES UNLIMITED[1]

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ABSTRACT

Recent surveys show that there are unlimited opportunities in the food, dairy, and regulatory agency fields. An entire new food product development program has started with the space age. It has become imperative to consider anew universal nutrition problems. Industry and educational institutions, as well as the individual himself have a responsibility to develop present opportunities through recruitment programs, training programs, research projects, and incentive plans. The future holds higher technological demands which must be developed to meet the food needs of the world's population.

Recent surveys show that there are unlimited opportunities in the food, dairy, and regulatory agency fields. One report states that *(1)* there are two jobs waiting for every graduate of an Agricultural College. There will be even more openings in business to process food for the growing population and to provide supplies, credit, and management services to farmers. Many job placement directors and hosts of food and dairy processors state that there are now at least three jobs available for every graduate specializing in the dairy and food processing fields. A similar man-power shortage exists in the regulatory agencies *(2)*.

An entire new food product development program has started with the space age. The future of the dairy, foods, and environmental sanitarian fields will offer many challenges which will mean the need for increased learning and a willingness to learn on the part of all concerned. How many people could have foreseen jobs for sanitarians from the lowly sowbean?

The new food product development program started with the space age has increased awareness of potential future problems unless we go "full speed ahead" with universal nutrition problems. In the very near future we will be faced with development of more and greater varieties of foods for outer space and other planet habitation as well as developing products from the sea. Our responsibilities will not stop with food development but will also include use of products to keep the atmosphere or environment clean, making products safe and free from contaminants for humanity, enforcement of clean air and soil conservation, development of safe chemicals and the testing of these, and water purification through proper sewage disposal.

Industry and educational institutions, as well as the individual himself have a responsibility to develop present opportunities through recruitment programs, training programs, research projects and incentive plans.

Upon checking with 12 of my associates with various dairy and food companies, I posed two questions. The answers I received were overwhelmingly the same. "Is there an opening in your organization for an agressive individual who wants unlimited opportunity to learn and progress provided his intelligence so dictates? Reply – "Yes, the opportunities for such an individual are unlimited." I followed with the question, what would you do to help this individual attain success? Reply – "Everything I could." The answers show a genuine interest by those in our industries and I am sure if the interest was directed to a potential recruit, more interest in our industries would be shown by the recruit.

TRAINING PROGRAM

To develop a recruit, to his fullest potential, a proper training program is a must. There are certain essential factors that lead to a successful training program as stated in a report to the College Relations Committee of the Milk Industry Foundation *(3)*.

(*a*) The training program must stem from top management. Unless the top management of the company is convinced that the training program is essential to the future progress of the company, it is almost certain to fail. Quality of personnel is of equal importance with quality in products.

(*b*) The training program must have specific objectives such as: (*i*) to increase the usefulness of technically trained personnel, (*ii*) to broaden the scope of the technically trained person, (*iii*) to overcome weaknesses in the trainee of which he may or may not be aware, (*iv*) to provide the trainee with a more extensive knowledge of business operations, (*v*) to give the trainee a thorough knowledge of the policies of the company, and (*vi*) to develop leadership for the company, the dairy-food industries, and the community.

(*c*) The program must be designed to give the trainee an opportunity to learn the operations of the

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entire plant. Many trainees have expressed dissatisfaction with being assigned a single routine task and left on that job indefinitely. This, obviously, indicates a lack of interest on the part of management, since little is being gained in training when the period of the assignment passes the point of diminishing returns.

(*d*) Duties should be assigned which will encourage the trainee to develop an analytical attitude to make him "cost conscious" and to stimulate him continually to ask "why?" Many times a trainee tends to draw conclusions without a background knowledge, particularly of cost.

(*e*) Regular reports should be required of the trainee.

(*f*) The supervisor of the trainee should make periodic reports.

(*g*) Reports must reach top management.

(*h*) Management should confer with the trainee.

(*i*) The training program should be broader than production alone.

(*j*) The program should include extra activities. One of the objectives of the program should be that of training for leadership. A characteristic of most leaders is that they do far more than is expected of them.

The cost of a training program is small but the dividends are great.

Interesting Students

A former college advisor of mine related that he wished there were some way that we could stimulate more interest on the part of young people to major in the dairy and food manufacturing fields. He stated that calls are received from industry in all parts of the country concerning open positions in the dairy and food fields but that the University can't fill them all. He further stated that the opportunities in the dairy and foods area are excellent but that the university has not been able to sell the high school and junior college students on this fact. The University has tried to promote a work study program in which the student would be able to earn the cost of his college education. Some companies have even promised to pay the student's tuition while attending school. Even this has not attracted a first inquiry on the part of the student. Copies of a folder stating the jobs that are available in the dairy and food industries have been sent to high schools and junior colleges but this has not yielded any response at this time.

If programs such as this have not generated any interest, where are we failing? It appears that the communication from high school students to the higher institutions of learning to industry remains a broken chain. Industry must get to the student at both levels of learning. Dairy and food plant executives and regulatory personnel should make contact with high school students at career days, stressing that the dairy and regulatory vocations require business, chemistry, biology, and engineering backgrounds. All of us must establish direct lines of communications with high school guidance counselors, stressing that opportunities in our industries are unlimited. *Perhaps the International Association of Milk, Food, and Environmental Sanitarians should appoint a few of its members to serve on a committee which would have a direct line to the state and high school advisors and counselors.*

Opportunity Created by Individual

To create unlimited opportunity is not 100% the responsibility of the educational instructors and industry. The individual himself, after chosing his field of endeavor, must share the major part of his destiny. In my opinion there are two types of opportunity, "given" opportunity and "taken" opportunity. "Given" opportunity refers to that opportunity which is expressly written, a specific guideline such as a job description. "Taken" opportunity is the little extra interest or initiative by the individual that opens the door to unlimited opportunities. A combination of "given" and "taken" responsibility will assure an individual of success in his chosen occupation.

The New Man on the Job

What are the feelings of the new man on the job in our professions? After talking with such men recently and reading published comments of several years ago, I have come to the conclusion that the gripes of today are the same as the gripes of yesteryear. Do these sound familiar to you?

(*a*) "Too much emphasis is placed on laboratory training at the university and not enough on how much labor costs should be per gallon of product."

(*b*) "There is too much intra-company bickering when there should be a common goal, that of profit."

(*c*) "There is not enough incentive offered to production people. Sales people can prove their ability by commission schedules."

(*d*) "The pay scale is too low for the responsibility accepted."

(*e*) "Management just won't communicate with me on decision making."

(*f*) "In our company we sometimes seem to be more interested in attracting new graduates than we

are in developing the college men we have in the organization."

(*g*) "Top management is so 'high and mighty' that I spend my time putting out the fires they create through peon treatment of the worker. Top management does not have to be disrespectful to the worker to attain desired results."

(*h*) "I can see I am buried and will never be recognized again unless I move on."

(*i*) "Too many dairy and foods companies pay the union labor nearly as much as the college graduate. The cost of a college education is not justified by the small increase in wages."

(*j*) "The industry on a local level could cooperate more with the universities on training programs, summer work and experimental work."

(*k*) "I believe that greater opportunities exist in other fields for college-trained men. I also believe that the 'cream of the crop' is being recruited by other interests."

(*l*) "I would suggest that the first thing industry can do to aid both the dairy-food graduate and itself, is to set up the appropriate training program for the individual concerned and follow it through to the end 'come hell or high water' making certain that the specified times of the program are strictly adhered to."

CONCLUSION

In conclusion, there are indeed unlimited opportunities in the food, dairy, and regulatory agency fields. With the advent of the space age, we have a responsibility to develop opportunities so that future universal nutrition problems can be resolved by competent, well trained people in our professions.

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THE ANNUAL CONVENTION OF THE BAKERY EQUIPMENT MANUFACTURERS ASSOCIATION—JUNE 16-23, 1974

The annual convention of the Bakery Equipment Manufacturers Association will be held at the new Southampton Princess Hotel, Southampton, Bermuda, June 16-23, 1974. Once again BEMA is holding its Annual Convention on this delightful island just about an hour-and-a-half by air from the States. This new and beautiful all air-conditioned hotel with all recreational facilities is designed to provide all in attendance healthful and relaxing periods interspersed throughout the work program of practical value.

The ABA-BEMA joint exposition committee will hold its final meeting at the Southampton Princess Hotel on Monday and Tuesday, June 17-18, 1974. All of Bakery Expo '73 activities will be finally reported, evaluated and recommendations made for the future.

The BEMA preliminary program features matters of interest and concern to every member of BEMA—such as—

The Baking Industry Exposition — The accomplishments of Bakery Expo '73, improvement suggestions, recommendations as to where, when and plans for the next Baking Industry Exposition. Factual reports will be made and the meeting will be open for general discussion.

Baking Industry Standard and BISSC Equipment Certification — Progressive revision of Standards, Certification procedure, promotion for broad understanding and usage.

Saftey — Bakery Safety Code and OSHA — Industry efforts and government recognition, and sound planning ahead.

AN AGAR MEDIUM FOR THE DIFFERENTIAL ENUMERATION OF YOGURT STARTER BACTERIA[1, 2]

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Abstract

Generally, *Streptococcus thermophilus* ferments sucrose, whereas *Lactobacillus bulgaricus* fails to utilize this disaccharide. When both sucrose and lactose were added to a basal medium, *S. thermophilus* fermented both carbon sources, produced sufficient acid to change the color of an acid-base indicator (bromcresol purple), and hence formed yellow colonies. On the same medium, most *L. bulgaricus* strains grew more slowly, produced less acid, and yielded white colonies. Acid diffusion around the *S. thermophilus* colonies was localized by incorporation of $CaCO_3$ into the medium. To test the efficacy of this medium when known strains of starters are used, the effect of freezing with liquid nitrogen on mixed cultures of *S. thermophilus* and *L. bulgaricus* was studied.

The sale of yogurt in the United States has increased almost six-fold in the past 10 years. In 1962, about 45.5 million lb of yogurt were sold; the figure for 1972 amounted to 288.8 million lb *(20)*. With the present emphasis on "health foods," weight-reducing diets, and development of flavored yogurts, consumption of this fermented milk product will continue to show an upward trend. The quality of yogurt is largely dependent on milk and additives used, starter culture added, cultural conditions, and subsequent handling of the product. With proper quality tests on raw materials used and with rigid control over sanitation and cultural conditions, many of the lapses and variables affecting product quality can be controlled. The one big variable, however, would be the starter culture. The modern dairy processor depends almost entirely on commercial culture manufacturers for the supply of suitable starters for cultured dairy products.

According to Davis et al. *(3)*, the commercial yogurt of today contains approximately equal proportions of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Kon *(8)* defined cultured yogurt as an acid product made with a specific symbiotic culture of *L. bulgaricus* and *S. thermophilus*. Humphreys and Plunkett *(6)*, in their extensive review of literature pertaining to yogurt, found that most investigators agreed that these two bacterial species are essential for production of yogurt. In the dairy industry, *S. thermophilus* is commonly referred to as "coccus," and *L. bulgaricus* as "rod." Symbiotic growth of *S. thermophilus* and *L. bulgaricus* in milk is now well established. Several studies have been conducted to elucidate the nature of this associative growth *(2, 10, 11, 19, 21)*.

There is considerable agreement among various investigators on the desirable ratio of coccus to rod in yogurt starters. Stocklin *(19)* recommended that the ratio of the rod to coccus should be maintained at either 1:1 or 1:1.2. Platt *(14)* described a successful process for yogurt manufacture, where equal proportions (in terms of inoculation rate, namely 2%) of separate milk cultures of coccus and rod are used. Pette and Lolkema *(12)* also reported that, in yogurt, a coccus:rod ratio of 1:1 is desirable after incubation and cooling. Sellars and Babel *(18)* recommended a similar ratio for yogurt starters. Schulz and Hingst *(17)* reported that for typical acetaldehyde flavor of yogurt, the coccus:rod ratio should be 3:1. Pette and Lolkema *(13)* further reported that the proportion of coccus and rod in the finished product is influenced by incubation temperature, acidity of the culture, and the rate of inoculum.

Because of the emphasis on maintenance of the desirable 1:1 ratio of coccus to rod for manufacturing good quality yogurt, there is a need for a suitable technique to determine the relative proportions of *S. thermophilus* and *L. bulgaricus* when grown together in milk cultures. Currently there is no simple, reliable method for the differential enumeration of the yogurt starter bacteria. Davis et al. *(3)* described an agar and a double pour-plating technique that allowed differentiation of coccus and rod by colony types under a low-power microscope. Selective media for lactobacilli have been described *(16)*, but use of

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two different media to obtain separate counts of coccus and rod strains in yogurt becomes cumbersome and, in some instances, unreliable *(3)*. Sellars and Babel *(18)*, Mocquot and Hurel *(9)*, and Johnson *(7)* suggested microscopic examination of yogurt cultures to roughly determine the ratio of coccus to rod, but by this technique dead bacteria cannot be distinguished from viable ones. So, the microscopic method also was considered inadequate *(3)*. The most recent attempt at developing a differential medium for these bacteria was reported by Porubcan and Sellars *(15)*. In this agar medium, differentiation was achieved by adding an appropriate sugar or sugar mixture (selected according to the carbohydrate fermentation characteristics of particular strains) to molten agar base before plating. On this agar, strains of *L. bulgaricus* produced diffuse, low mass colonies (2 to 10 mm in diameter), and *S. thermophilus* strains grew as discrete, dense colonies (1 to 3 mm in diameter). For routine use by personnel with limited training, however, this medium has the same limitation as the one proposed by Davis et al. *(3)* in that differentiation is based on an unreliable criterion; namely, colony morphology.

This paper describes an agar medium developed in our laboratory for differential enumeration of yogurt starter bacteria. In developing this medium, we carefully studied the carbohydrate fermentation patterns of several coccus and rod strains. All *S. thermophilus* strains fermented lactose and sucrose; the rod strains fermented lactose, but most failed to utilize sucrose. When a suitable combination of sucrose and lactose was provided, the rate of acid production by *S. thermophilus* was enhanced, when only a very limited amount of utilizable sugar (lactose) for *L. bulgaricus* was made available, acid production by the rod was restricted. The amount of lactose, however, was adjusted to afford sufficient growth of the rod to form good colonies on the agar. To accentuate differences in fermentation rates of available carbohydrates, incubation conditions were carefully standardized. For visual detection of differences in fermentation rates, bromcresol purple, a pH indicator sensitive within the specific pH range encountered in this system, was incorporated into the medium. Relatively insoluble, uniformly suspended $CaCO_3$ was included in the agar to prevent acid diffusion throughout the medium. This, in effect, localized the acid produced by individual colonies.

To demonstrate a practical application for this agar, the effect of liquid nitrogen (LN_2) freezing on the relative proportions of the component strains in a milk culture containing both coccus and rod was determined by differential enumeration.

MATERIALS AND METHODS

Cultures

Ten strains of *S. thermophilus* and nine of *L. bulgaricus* were studied in this investigation (Table 1). The cultures were available in the collection at the Department of Food Technology, Iowa State University. All cultures were maintained by twice-a-week transfer in sterile, reconstituted 11% nonfat milk. They were grown at 32 C for 18 h and held at 5 C between transfers.

Composition of the agar and plating technique

The composition of the differential agar (hereafter referred to as Lee's agar) is as follows: Tryptone 1.0%; yeast extract 1.0%; lactose 0.5%; sucrose 0.5%; $CaCO_3$ 0.3%; K_2HPO_4 0.05%; bromcresol purple (BCP) 0.002%; and agar 1.8%. The pH of the medium before sterilization (121 C for 20 min) was adjusted to 7.0 $\pm$ 0.1. Because of the minute requirement of BCP in the agar, this ingredient was added in the form of 1.0 ml of sterile 0.2% solution (autoclaved at 121 C for 15 min) per 100 ml of sterile agar just before pouring petri plates.

After addition of BCP to the sterile, melted medium, the agar was thoroughly mixed to evenly suspend the settled $CaCO_3$; care was taken to avoid excessive incorporation of air. Agar was then poured into previously chilled, sterile petri plates to obtain a layer 4 to 5 mm thick. Chilling of petri plates (in a refrigerator for 30 min) facilitated rapid gelation of the agar, thus insuring uniform distribution of $CaCO_3$ in the agar layer. After the agar solidified, plates were dried in a 30 C incubator for 18-24 h.

Coagulated single-strain milk cultures of *S. thermophilus* and *L. bulgaricus* and various two-strain combinations containing one strain each of coccus and rod were diluted to 1×10^{-6} according to *Standard Methods for the Examination of Dairy Products (1)*. Samples (0.1 ml) of the dilutions were evenly spread on the surface of the agar layer with a sterile bent glass-rod. The plates were incubated in a CO_2-incubator at 37 C for 48 h.

To compare the merits of Lee's agar in terms of efficiency of recovery of the coccus and rod microorganisms (measured as CFU/ml), dilutions of the various single-strain and combination cultures were similarly spread on a parallel set of lactic agar plates *(4)* and incubated exactly alike.

To study the effect of LN_2 feezing on the relative proportions of coccus and rod strains in a mixed yogurt culture, *S. thermophilus* D and *L. bulgaricus* 7 were grown separately in sterile, reconstituted 11% nonfat milk for 16 h at 37 C. At the end of incubation, cultures were added together at 1:1 vol/vol ratio and thoroughly mixed; 1.0 ml of the mixture was inoculated into 100 ml sterile, reconstituted 11% nonfat milk. After incubation at 37 C for 6 h, a portion of the culture was aseptically removed, and the remaining portion was incubated further for 6 h. The first portion was immediately plated on Lee's agar and tested for acid-producing activity *(5)*. At the same time, 3.0 ml of the culture was transferred into a sterile, screw-cap test tube (16 mm $\times$ 125 mm), plunged into a mixture of LN_2, and held there for 2 weeks. At the end of the 12-h incubation period, the remaining portion of the culture was subjected to similar treatments. After 2 weeks, tubes were removed; the contents were thawed in a 37 C water-bath, plated on Lee's agar, and tested for acid-producing activity.

RESULTS AND DISCUSSION

In the development of Lee's agar, first a detailed investigation was done with various strains of *L. bulgaricus* in pure cultures. These experiments were directed at establishing the critical amount of lactose necessary in the formulation to obtain good colonial growth of the rod, without causing the indicator color change from neutral (violet) to acid (yellow) range. In these trials, the amounts of lactose were varied within the narrow range of 0.4% to 0.5%. Even at a concentration of 0.48% lactose in the formula, colonial growth of lactobacilli was poor. At 0.5% level, good colonial growth was obtained. At the same time, this concentration was restrictive enough to prevent sufficient acid production by the lactobacilli to cause the change in indicator color under the specific incubation conditions used. Hence, lactose concentration was fixed at 0.5% in the final formulation of the agar. All the *L. bulgaricus* strains included in this study (Table 1), when plated on the medium made according to the described formula and incubation under the recommended conditions, appeared as white colonies. With prolonged incubation, however, the lactobacilli accumulated sufficient acid to shift the indicator color toward yellow. So, for obtaining differentiation of coccus and rod, the recommended incubation conditions should be strictly followed.

Because of the availability of two utilizable sugars (lactose and sucrose) in the medium and the favorable incubation conditions used, *S. thermophilus* grew rapidly and produced enough acid to change indicator color. This was readily seen by the acid-induced yellow color of the coccus colonies. Uniformly suspended $CaCO_3$, acting as a nondiffusible buffer, localized the acid, producing a narrow intense yellow zone around individual *S. thermophilus* colonies.

When mixtures of *S. thermophilus* and *L. bulgaricus* grown in milk were plated on this agar and incubated under the specified conditions, the coccus produced yellow colonies, and the rod appeared as white colonies. Also, Lee's agar compared favorably with lactic agar *(4)* for enumerating efficiency. The data are presented in Tables 1 and 2. Because of the close agreement in counts between the two media, no statistical analyses were made.

To illustrate a practical application for Lee's agar, the effect of freezing and storage in LN_2 on the relative cell numbers of *S. thermophilus* D and *L. bulgaricus* 7, when grown together in milk, was investigated. Results are summarized in Table 3. The data indicate that a young milk culture (6-h old) could be successfully preserved in LN_2 with-

TABLE 1. EFFICIENCY OF RECOVERY OF *S. thermophilus* AND *L. bulgaricus* IN SINGLE-STRAIN MILK CULTURES ON LEE'S AND LACTIC AGAR

Species	Strains	Lactic agar (spread plate)	Lee's agar (spread plate)
		(counts × 10^7)	
S. thermophilus strain[a]	A	53	yellow 51
	B	31	yellow 40
	C	47	yellow 58
	D	36	yellow 30
	E	69	yellow 78
	F	40	yellow 20
	G	75	yellow 72
	H	53	yellow 75
	I	65	yellow 69
	J	80	yellow 78
L. bulgaricus strain[a]	1	38	white 50
	2	71	white 60
	3	17	white 14
	4	77	white 62
	5	56	white 92
	6	106	white 110
	7	44	white 45
	8	98	white 105
	9	103	white 122

[a]All strains studied were obtained from commercial sources.

TABLE 2. EFFICIENCY OF RECOVERY OF *S. thermophilus* AND *L. bulgaricus* IN MIXED-STRAIN COMBINATIONS ON LEE'S AND LACTIC AGAR

Mixed-strain combination	Lactic agar (spread plate)	Lee's agar (spread plate)	Total count on Lee's agar
	(counts × 10^7)		
C + 5	41	yellow 22 white 24	46
E + 5	66	yellow 22 white 46	68
D + 4	62	yellow 23 white 38	61
I + 8	143	yellow 36 white 130	166
J + 9	67	yellow 41 white 44	85
D + 7	42	yellow 23 white 33	56
B + 2	28	yellow 32 white 10	42
F +6	47	yellow 19 white 44	63
C + 2	8	yellow 2 white 13	15
E + 7	32	yellow 20 white 21	41
D + 1	82	yellow 46 white 45	91
H + 6	71	yellow 46 white 62	108
A + 5	42	yellow 50 white 4	54
G + 3	30	yellow 49 white 9	58
D +2	105	yellow 67 white 50	117

TABLE 3. EFFECT OF FREEZING AND STORAGE AT −196 C ON THE VIABILITY AND ACID-PRODUCING ACTIVITY OF YOGURT STARTER MIXTURE

	6-h Culture		12-h Culture	
	Counts × 10^7	TA[a]	Counts × 10^7	TA
Before freezing in LN_2	yellow 91 white 22	0.82%	yellow 80 white 22	0.82%
After frozen storage in LN_2	yellow 123 white 30	0.81%	yellow 6 white 51	0.82%

[a]TA = Titratable acidity expressed as percent lactic acid.

out the loss of strain viability or acid-producing activity. An increase in cell numbers after freezing (Table 3) could have been caused by the breakage of chains during freezing and subsequent thawing, and this phenomenon was observed in several instances in our investigation. After 12 h at 37 C, because of the excessive accumulation of lactic acid, S. *thermophilus* D had probably reached the phase of acid-injury and decline. This probably accounts for the dramatic loss of viability of the coccus strain after frozen storage. Because of the relatively greater acid tolerance of the rod, there was no loss in viability. A major portion of the acid-producing activity of the 12-h culture after frozen storage could be attributed to the lactobacilli. In related experiments (data not presented here), however, we observed that the loss of viability among component strains in mixed yogurt starters varied from strain to strain and also was influenced by the age of the culture at freezing. Subculturing colonies picked from plates verified the relationship between colony color and classification.

In the discussion of the merits of this medium, certain of its inherent limitations should be mentioned. Because differentiation on this agar is based on acid-producing activity, restriction of acid diffusion within a small area, and its visualization by a pH indicator, a preponderence of either S. *thermophilus* or lactobacilli in a mixture does not allow distinction between the rod and coccus colonies. The most desirable results are obtained when the proportions of the coccus and the rod are fairly equal and when the total number of colonies on the plate does not exceed 250. Because of this limitation, Lee's agar is not recommended for general application in determining differential counts of S. *thermophilus* and *L. bulgaricus*, for example, in starter mixtures for Italian cheeses or in certain hard cheeses themselves (Italian and Swiss varieties) where S. *thermophilus* and *L. bulgaricus* are found in various proportions. In these instances, however, this agar could be used if proper judgment is exercised as to the age of the culture or the cheese, etc. Obviously, this is not a selective medium, and many other microorganisms would be expected to grow rapidly on it, with and without production of acid. Another limitation pertains to the incubation conditions, which are quite specific to obtain the best results. Finally, and perhaps most importantly, we have found considerable strain variation among species of *L. bulgaricus* in regard to their acid-producing activity on this medium. Many strains can form yellow colonies virtually indistinguishable from those developed by S. *thermophilus*. This difficulty can be obviated by the use of known, pretested strains in the starter mixtures.

The agar we have described here has specific application for yogurt. Several commercial yogurts were successfully tested for differential counts on Lee's agar in our laboratory. Lee's agar would aid in the quality control of freshly prepared yogurt starters in ensuring the availability of the desired ratio of coccus to rod. Also, the effect of various starter preservative techniques (lyophilization, freezing at −20 C or −196 C) on the optimum ratio of strains could be determined. From this, the most suitable preservative technique for any specific yogurt starter can be selected. Finally, in the large-scale manufacture of yogurt, the exact effects of the various cultural and processing conditions on the final balance of coccus and rod in the finished product could be determined. This would help in process standardization to obtain the most desirable ratio of the starter strains in the end-product, a factor usually associated with top quality yogurt *(12)*.

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AOAC WILL HOLD OCTOBER MEETING

The Association of Official Analytical Chemists will hold its 88th Annual Meeting October 14-17, 1974, at the Marriott Hotel, Twin Bridges, Washington, D. C. The latest developments in analytical methodology for many commodities and materials important to agricultural and public health areas will be presented and discussed.

About 1,400 chemists, microbiologists, physicists, and their administrators will attend, representing national, state, provincial, and local government agencies, universities, and industries in North America and elsewhere. Over 240 papers will be given on new techniques, methods, and instrumentation for analysis of drugs, feeds, fertilizers ,foods, food addities, pesticides, flavors, beverages, microbiological contamination of foods, mycotoxins, and related subjects.

A special symposium is being planned on toxicological methodology. Tests for safety evaluation of substances and consideration of certain toxicological tests for standardization which are important to regulatory agencies are planned for discussion. Also the Society of Cosmetic Chemists and the AOAC will hold a joint symposium on cosmetic analytical techniques Monday afternon, October 14th. Three simultaneous scientific sessions will be held Monday afternoon through Thursday morning on about sixty subject areas named or related to those given above. Additionally evening workshops are being planned on automated analyses and thin layer chromatography. A highlight of the banquet Monday evening will be the presentation of the Harvey W. Wiley Award to a scientist for his outstanding contribution to analytical methodology. Musical entertainment will be provided for the pleasure of those at the banquet.

Nearly 40 firms will exhibit the latest laboratory equipment and supplies. Registration will continue from 1:00 p.m. Sunday, October 13, through Thursday morning, October 17. The registration fee will be $5.00 for one day or $10.00 for 2 or more days. Anyone interested is invited to attend. For further information, please contact Luther G. Ensminger, Association of Official Analytical Chemists, Box 540 Benjamin Franklin Station, Washington, D. C. 20044.

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PREPARATION AND EVALUATION OF A MICROBIOLOGICAL GROWTH MEDIUM FORMULATED FROM CATFISH WASTE PEPTONE

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Abstract

A water-soluble, heat-stable peptone was extracted from enzymatically digested channel catfish (*Ictalurus punctatus*) heads and skins. Proximate analyses showed that the peptone was composed of 89.02% protein (Kjeldahl N × 6.25), 10.42% ash and 0.44% fat. The peptone contains all amino acids demonstrated to be present in concentrations of greater than 1.0% in six other commercially available microbiological peptones used for comparative purposes in the study. Total cell mass production on catfish and test peptone media by microorganisms representing 13 genera was measured. Catfish peptone supported luxuriant growth of the test organisms and an accumulative average of dry cell mass weights produced by the organisms revealed that the catfish peptone ranked third out of the seven peptones tested. This new peptone offers the clinical and industrial microbiologist a potentially valuable ingredient for formulation of growth and fermentation media.

Exploration of protein hydrolysates from fish and fishery by-products for microbiological media application is limited but not new. Tarr and Deas *(18)* demonstrated that tryptic digests of various fish species were excellent nutrient components for growth of certain anaerobes and Snieszko *(11)* used a fish peptone to prepare an enrichment medium for a fish pathogen. Krishnaswamy and Lahiry *(7)* reported on the mircrobiological evaluation of papaic digests of freshwater fish flesh. More recently Green et al. *(5)* showed that fish peptones compared favorably to commercially available peptones and standard media in their ability to support microbial growth. According to Strasdine and Melville *(15)*, salmon-canning waste water will support growth of bacteria both as a complete growth medium and as a supplementary source of available nitrogen. The mass of research effort in the area of fish hydrolysis has not been directed toward its utilization in growth media, however, but rather toward evaluating methodology for the preparation of hydrolysates for use as foods and feeds *(4, 6, 8, 10, 12, 13, 14, 16, 17)*.

Twelve million pounds of farm-raised channel catfish (*Ictalurus punctatus*) were projected to be slaughtered and processed in the United States in 1973 *(9)*. By-products from catfish processing amount to 40-45% of the live weight of fish purchased. Approximately half of the nearly four million pounds of waste will be sold at 2-3 cents per pound with only a portion of this material utilized as fishmeal. The remaining two million pounds of waste must be properly disposed of at the expense of the processor. Realizing that on a wet weight basis 6-8% of catfish waste is protein, an experiment was designed to evaluate the feasibility of extracting water-soluble peptones from catfish waste for use in the formulation of microbiological growth media. This paper reports a procedure for preparation of a heat-stable, water-soluble catfish peptone from catfish heads and skins using proteolytic enzymes. Data from studies involving the comparison of catfish peptone to six commercially available peptones for its ability to support the growth of microorganisms and its potential use as an ingredient in microbiological growth media are presented.

Materials and Methods

Preparation of catfish peptone

Channel catfish heads and skins were collected from freshly slaughtered carcasses and combined with water at a 1:1:1 ratio (heads:skins:water). Bromelain (Miles Laboratories, Inc., Marschall Division, Elkhart, Ind.) was added to the mixture to a concentration of 0.02% based on the wet weight of waste. Proteolysis was allowed to progress, with occasional stirring, over a 3-h period in a steam-jacketed kettle maintained at 50 C. Initial pH of the digest was 6.8. Sufficient 3 N HCl was then added to reduce the pH to 2.8 and a second proteolytic enzyme, Milezyme AFP (Miles Laboratories), was added at 0.02% based on the initial weight of waste in the digest. After an additional 3 h of hydrolysis at 50 C, the temperature of the mixture was increased to 80 C and maintained for about 5 min to inactivate the added enzymes and precipitate some of the less heat-stable proteins. Bones were mechanically removed from the liquified catfish waste and fat was skimmed from the top of the digest after cooling to 2 C overnight.

Solid material was separated from the liquified waste by centrifugation at 10,000 × g and discarded. The supernatant fluid was adjusted to pH 6.8 by the addition of 2 N NaOH and solids were again removed after centrifugation at 10,000 × g. The supernatant fluid was filtered (Whatman No. 1) at 24 C using a Buchner funnel and water-soluble peptones were concentrated in a Mojonnier Lo-Temp Evaporator (Model LTFL, No. 43, Mojonnier Bros. Co., Chicago). Product temperature did not exceed 29 C during this process. Upon reaching a four-fold concentration, the liquid was frozen at −70 C, freeze-dried, and pulverized. The end product is referred to below as catfish peptone.

TABLE 1. COMMERCIALLY AVAILABLE PEPTONES USED IN COMPARATIVE STUDIES WITH CATFISH PEPTONE

Name	Lot no.	Substrate	Hydrolysis
Bacto-Peptone[a]	582639	Meat	Enzyme
Myosate[b]	809619	Heart muscle	Pancreatin
Thiotone[b]	301696	Animal tissue	Pepsin
Phytone[b]	710665	Soya meal	Papain
Trypticase[b]	708632	Casein	Pancreatin
XM-8[c]	081731	Atlantic Menhaden	Process unknown

[a]Difco Laboratories, Detroit, Michigan.
[b]Baltimore Biologicals Ltd., Cockeysville, Maryland.
[c]Zapata Haynie Corporation, Baltimore, Maryland.

Chemical and physical analyses of peptones

Protein *(1)* (Kjeldahl nitrogen × 6.25), ash *(1)* and fat *(3)* content in catfish peptone and six other commercially available peptones were determined. Moisture was determined by difference after drying 5-g samples under vacuum overnight at 70 C. Commercially available peptones examined were derived from red meat, fish, dairy, and vegetable sources and were included in the study for means of comparison to the catfish peptone. Table 1 lists the commercial peptones studied, their substrate origins, lot numbers, and manufacturers.

Amino acid analyses of all peptones were done on a Durram Amino Acid Analyzer, Model D-500 after acid hydrolysis of the protein according to the manufacturer's recommended procedures *(2)*.

Resistance of 0.5% catfish peptone solutions to coagulation at 121 C for 15 min was tested over a pH range of 2.0 to 9.0.

Evaluation of peptones in microbiological growth media

Catfish peptone and the six peptones listed in Table 1 were incorporated into media and evaluated for their ability to support growth of microorganisms representing 13 genera. Seven bacteria and six fungi were examined. The bacterial growth medium, with the exception of that used for *Vibrio* parahaemolyticus which contained an additional 3% NaCl, was formulated from 0.5% peptone and 0.1% dextrose in distilled water and was adjusted to pH 7.0. Each medium was dispensed in 50-ml aliquots into 250-ml Erlenmeyer flasks, stopped with cotton and autoclaved at 121 C for 15 min. *Escherichia coli, Salmonella enteritidis, Pseudomonas fluorescens, Staphylococcus aureus, Streptococcus faecalis,* and *Bacillus subtilis* were cultured at 30 C in nutrient broth (Difco) for 24 h and then diluted 1:100 in Butterfield's phosphate buffer. *V. parahaemolyticus* was cultured in tryptic soy broth (Difco) containing 3% NaCl and diluted in distilled water containing 3% NaCl. The diluted cultures were inoculated in 0.1-ml quantities into the test media and incubated at 30 C on a rotary shaker (150 rpm) for 24 h. Cells were collected by centrifugation at 9.000 × g, resuspended in 0.85% NaCl, and collected once again. Cells were then suspended in distilled water and quantities were dried in a forced-air oven overnight at 70 C before weighing.

Media for fungal studies contained 0.5% peptone and 1.0% dextrose and were adjusted to pH 5.5 with HCl. Fungi included in the study were *Aspergillus oryzae, Neurospora sitophila, Mucor heimalis, Actinomucor elegans, Rhizopus oligosporus,* and *Saccharomyces cerevisiae.* Spore and cell suspensions in water were prepared for 5-day old cultures grown on potato dextrose agar (Difco); 0.1 ml of the suspensions were individually added to the test media and incubated as described above for bacteria. The entire mat was collected from the filamentous fungi, washed with saline and water, then dried and weighed. Quantitation of *S. cerevisiae* cells was made according to the procedure described for bacteria.

Bacterial colony size, morphology, and pigmentation were observed after surface-plating 24-h old cultures on peptone media prepared as described earlier but containing 1.5% agar. Observations were made after incubation times of 24 h at 36 C and 96 h at 21 C.

RESULTS AND DISCUSSION

Preparation of catfish peptone is summarized in Fig. 1. Viscera were not included in the raw substrate because of their high fat content and inclusion of feed and fecal matter. Initial digestion with bromelain was at pH 6.8. This pH is in the optimum range for activity of bromelain and also is suitable for proteolysis by autolytic enzymes naturally present in the catfish waste. Milezyme AFP is a fungal protease having a temperature optimum of 50 C at pH 2.5. Further liquefaction of the waste was noticeable upon addition of Milezyme AFP, indicating substantial hydrolytic action on protein components in the di-

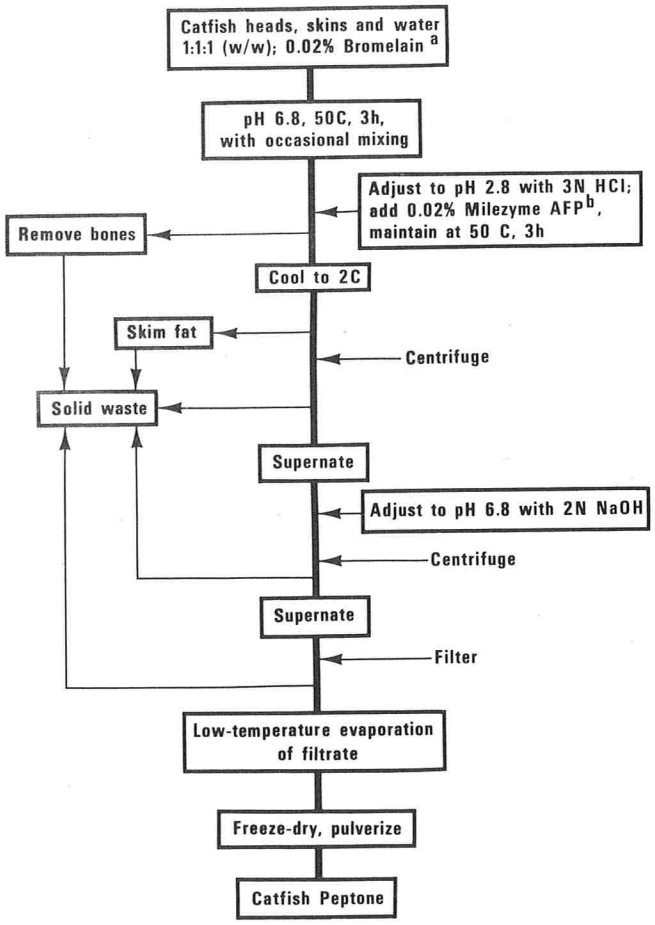

Figure 1. Separation of water-soluble catfish peptone from nonsoluble catfish head and skin constituents. [a]Bromelain is a proteolytic enzyme preparation isolated from pineapple stems and [b]Milezyme AFP is an acid fungal protease. Both enzymes were supplied by Marschall Division, Miles Laboratories, Inc.

TABLE 2. PROXIMATE COMPOSITION OF PROTEIN HYDROLYSATES USED IN MICROBIOLOGICAL GROWTH MEDIA

Component	Hydrolysate						
	Bacto-Peptone	Myosate	Thiotone	Phytone	Trypticase	XM-8	Catfish peptone
Protein[a], %	94.75	76.45	78.64	54.97	74.84	72.80	89.02
Ash, %	5.91	1.48	9.20	11.91	9.93	22.88	10.42
Fat, %	.06	.12	.12	.04	.12	.38	.44
Moisture, %	.99	2.39	3.87	5.31	2.77	5.01	1.00
Total	101.71	80.44	91.83	72.23	87.66	101.07	100.88

[a]Kjeldahl nitrogen × 6.25.

TABLE 3. AMINO ACID COMPOSITION[a] OF PROTEIN HYDROLYSATES USED IN MICROBIOLOGICAL GROWTH MEDIA

Amino acid and ammonia	g Amino acid per 100 g protein[b]						
	Bacto-Peptone	Myosate	Thiotone	Phytone	Trypticase	XM-8	Catfish peptone
Aspartic acid	7.72	10.81	9.30	13.68	7.26	9.18	7.85
Threonine	2.52	5.18	4.29	4.43	4.30	4.06	3.54
Serine	3.89	4.30	4.69	5.44	5.48	4.30	4.40
Glutamic acid	12.38	18.56	14.35	24.73	23.83	17.85	12.47
Proline	7.93	—[c]	—	—	5.99	—	5.47
Glycine	26.24	7.98	12.62	4.66	2.08	24.04	23.07
Alanine	11.00	7.93	8.66	4.51	3.13	16.40	10.87
Valine	3.43	6.15	6.68	4.86	6.84	6.42	3.70
Methionine	—	1.88	1.74	—	2.45	—	1.02
Isoleucine	2.33	5.31	4.07	4.56	5.52	4.08	2.58
Leucine	4.17	9.80	8.79	7.36	9.62	7.46	4.49
Tyrosine	1.07	1.35	2.44	2.59	2.67	1.40	1.45
Phenylalanine	2.98	5.37	5.29	5.12	5.82	4.43	3.21
Histidine	1.00	2.58	2.12	2.62	2.83	—	1.61
Lysine	4.58	9.07	7.13	6.84	8.16	—	5.17
Ammonia	.32	1.15	.80	1.57	.79	.37	.77
Arginine	8.55	2.57	7.02	7.01	3.22	—	8.31

[a]Tryptophan not determined.
[b]Kjeldahl nitrogen × 6.25.
[c]Indicates less than 1% of protein.

gest. Adjustment to pH 6.8 later in the processing scheme resulted in significant quantities of insoluble protein. This was expected since the isoelectric point of the catfish waste protein is probably in the pH range of 5 to 6 as indicated by Meinke and Mattil (8) for whole carp protein. Separation of protein which is insoluble at pH values generally observed in microbiological growth media was a desirable step in the preparation of peptone. Although the end product of the scheme shown in Fig. 1 is referred to as "catfish peptone," it should be noted that the presence of free amino acids is likely. Analyses were not done to categorize or quantitate hydrolyzed waste protein with respect to peptide length.

Proximate compositions of all protein hydrolysates examined in the study are shown in Table 2. Total nitrogen as measured by the Kjeldahl technique was expressed in terms of percent protein using 6.25 as a conversion factor, regardless of the origin of the protein contained in the peptones. No attempt was made to identify primary and secondary protease nitrogens, peptone nitrogen, and free amino acid nitrogen fractions in the hydrolysates. Catfish peptone ranked second only to Bacto-peptone in nitrogen content. Ash varied from 1.48% in Myosate to 22.88% in XM-8. Adjustment of pH first with HCl and then with NaOH during preparation undoubtedly elevated the ash contents of some of the hydrolysates, including the 10.42% found in catfish peptone. Mechanical separation rather than solvent extraction was employed to remove fat from liquefied catfish waste. Although the catfish peptone contained 0.44% ether-extractable material (fat), which was the highest amount in any of the peptones analyzed, this level did not interfere with the clarity of 0.5% peptone solutions. Moisture content was variable due to the extreme hygroscopicity of the hydrolysates.

Amino acid analysis data are presented in Table 3. Substantial levels of proline and glycine in catfish peptone indicate digestion of collagen during its preparation. Catfish peptone contains all amino acids found at concentrations of greater than 1.0% in the commercial peptones included in the study. Availability of the acids was not measured by bioassay procedures.

Ability of protein hydrolysates to support growth

TABLE 4. DRY CELL MASS YIELD FROM MICROORGANISMS CULTURED IN MEDIA PREPARED FROM PROTEIN HYDROLYSATES

Microorganism	mg of Cells per 100 ml medium						
	Bacto-Peptone	Myosate	Thiotone	Phytone	Trypticase	XM-8	Catfish peptone
Bacteria							
Escherichia coli	72	183[a]	175[b]	109[c]	93	47	96
Salmonella enteritidis	30	80[a]	55[b]	17	42	50[c]	47
Vibrio parahaemolyticus	148	149[c]	185[b]	110	121	189[a]	140
Pseudomonas fluorescens	107	192[a]	182[b]	124	129	69	144[c]
Staphylococcus aureus	11	75[a]	54[b]	32	9	19	43[c]
Streptococcus faecalis	23	45[a]	40[c]	41[b]	13	30	38
Bacillus subtilis	26	52	44	155[a]	92[b]	65[c]	47
Fungi							
Aspergillus oryzae	189[c]	152	337[a]	122	94	111	224[b]
Neurospora sitophilia	147[c]	118	251[a]	119	74	71	155[b]
Mucor hiemalis	120	274[b]	275[a]	147	187	76	233[c]
Actinomucor elegans	144	279[c]	335[a]	219	208	127	285[b]
Rhizopus oligosporus	133	145[c]	215[a]	132	120	41	154[b]
Saccharomyces cerevisiae	27	397[a]	337[b]	264	31	108	321[c]
Average (all microorganisms)	91	165[b]	191[a]	122	93	77	148[c]

[a,b,c]Weights of dry cell mass ranked as highest, second highest and third highest, respectively, produced by each microorganism in protein hydrolysate media.

of bacteria and fungi was measured by determining total cell mass production on the various peptone-dextrose media. Results are presented in Table 4. Organisms tested exhibit a wide range of metabolic activities and morphological characteristics. Although catfish peptone did not rank first with respect to cell mass production for any of the 13 organisms tested, it consistently ranked high; an accumulative average showed that catfish peptone ranked third out of the seven peptones tested. Thiotone and Myosate were first and second, respectively.

Bacterial colony size and pigmentation varied somewhat on solid media containing different hydrolysates, however no striking dissimilarities were noted. Colony development and numbers were similar for specific media regardless of incubation at 21 or 36 C.

It is not by coincidence that procedures outlined for production of catfish peptone are optimum for efficient recovery of the highest grade product. Chemical and physical manipulation of digestion and concentration processes would undoubtedly yield higher quantities of perhaps higher quality peptone. Nevertheless, data obtained from these experiments demonstrate that a water-soluble, heat-stable peptone can be produced from catfish waste. The peptone has a respectable amino acid profile and competes favorably with commercially available peptones in supporting the growth of bacteria and fungi. Catfish peptone offers the clinical and industrial microbiologist a new, potentially valuable ingredient for preparation of growth and fermentation media.

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Louis A. King — Henry V. Atherton

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Registration and Speakers Hospitality: Jay Boosinger, 3418 N. Meridian Rd., Tallahassee, Florida 32303
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Publicity: Dick Jolley, 5000 Argon Way So., St. Petersburg, Fla.
Bronson Lane, P. O. Box 7813, Orlando, Fla. 32804

Door Prizes: Ralph Kirkland, P. O. Box 3384, Tampa, Florida 33601
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Program & Meeting Room Arrangements: DOC HATCHER, P. O. Box 1111, Tampa, Florida 33601

AFFILIATE COUNCIL OFFICERS

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AFFILIATE REPRESENTATIVES

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HOWARD HUTCHINGS	South Dakota
W. H. GILL	Virginia
L. O. LUEDECKE	Washington
WAYNE BROWN	Wisconsin

SUNDAY, AUGUST 11, 1974

1:00- 8:00 p.m.—Registration—Arcade
1:30- 5:30 p.m.—Executive Board—Coquina Key
6:00- 7:00 p.m.—Early Bird Reception—Hilton Room
8:00-11:00 p.m.—Executive Board—Coquina Key

MONDAY, AUGUST 12, 1974

8:00 a.m.- 8:00 p.m.—Registration—Arcade Message Center

SPECIAL MEETINGS

9:00 a.m.-12:00 noon—Executive Board—Coquina Key

1. Report on Local Arrangement
2. Report of Executive Secretary
3. Report of Sanitarians Joint Council

1:00- 3:00 p.m.—Membership Committee—Tampa Bay

1:00- 3:00 p.m.—Affiliate Council—South Ballroom Section

1. International Program
2. Discussion of Association Awards
3. Affiliate Input to Journal of Milk and Food Technology
4. Discussion of possible IAMFES and NEHA Merger
5. Future Meeting Location Recommendations
6. Election of Affiliate Council Officers

1:30- 5:00 p.m.—Executive Board—Coquina Key

1. Report of Journal Management Committee
2. Regular Agenda
3. Committee Chairman
4. Report of Affiliate Council
5. Meet with Past Presidents

1:30- 5:00 p.m.—Individual Committee Meetings—South Ballroom Section
(See Bulletin Board—Arcade)

3:00- 5:00 p.m.—Farm Methods Committee—South Ballroom Section

6:00- 7:00 p.m.—Reception—Lucayan Room

8:30-10:00 p.m.—Discussion of 14th Edition of Standard Methods—South Ballroom
ELMER H. MARTH
ROBERT MARSHALL

FLORIDA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS ANNUAL MEETING BAY A & B

8:45 a.m.—ADDRESS OF WELCOME
SAM NOLES,
President of FAMFES

9:00 a.m.–STATUS OF THE UNIVERSAL PRODUCT CODE
B. C. STURGIS,
Weyerhauser Co.

9:45 a.m.–NATIONAL POLLUTION DISCHARGE ELIMINATION SYSTEM–IMPACT ON AGRICULTURE
L. B. BALDWIN,
University of Florida

10:30 a.m.–MILK BREAK
North Arcade

11:00 a.m.–EFFICIENCY FACTORS IN LABORATORY QUALITY CONTROL
ROY GINN,
Minnesota Dairy Quality Control Institute, Inc.

12:00 Noon–AWARDS LUNCHEON–FAMFES
North Ballroom

1:45 p.m.–METHODS FOR REDUCING IN-PLANT LOSSES
DALE A. SEIBERLING,
Economics Laboratory, Inc.

2:30 p.m.–BUSINESS MEETING

TUESDAY, AUGUST 13, 1974

8:30 a.m.- 5:00 p.m.–REGISTRATION–Arcade
Message Center Cloakroom

8:00 a.m.- 9:00 a.m.–EXECUTIVE BOARD
Coquina Key

MORNING—GENERAL SESSION
GRAND BALLROOM

PARNELL J. SKULBORSTAD
President Elect, Presiding

9:30 a.m.–INVOCATION
DR. C. BRONSON LANE

9:35 a.m.–ADDRESS OF WELCOME
JULIAN LANE,
State Senator, Florida

10:15 a.m.–PRESIDENTIAL ADDRESS
EARL O. WRIGHT

10:45 a.m.–NDC NUTRITION RESERCH PROGRAM AND ITS RELATIONSHIP TO A NATIONAL POLICY
ELWOOD W. SPECKMANN

11:45 a.m.–NOMINATIONS, 1974

AFTERNOON—MILK SANITATION SECTION
GRAND BALLROOM

HENRY V. ATHERTON, Presiding

1:30 p.m.–DOOR PRIZE DRAWING

1:45 p.m.–CONTROL OF MILK QUALITY IN RETAIL MARKETS
WARD PETERSON

2:05 p.m.–MILK QUALITY IN THE PUBLIC SCHOOL SYSTEM
DAVID K. BANDLER

2:50 p.m.–THE IRON CONTENT OF SEPARATOR AND CLARIFIER SEDIMENT
B. J. DEMOTT

3:15 p.m.–MILK BREAK
North Arcade

3:30 p.m.–EXPERIENCE IN MILK DATING
JOE R. ANTINK
JOHN SPEER
JOHN C. BRUHN

4:15 p.m.–BULK MILK HANDLING PROCEDURES
RAY A. BELNAP

4:45 p.m.–PRELIMINARY STUDY TO DETERMINE THE FEASISILITY OF USING A 0.20 INCH DIAMETER DISK TO MEASURE SEDIMENT IN FLUID MILK
EARL O. WRIGHT
DONALD K. HOTCHKISS
WARREN S. CLARK, JR.

AFTERNOON—FOOD SANITATION SECTION
BAY A

HAROLD E. THOMPSON, Presiding

1:30 p.m.–DOOR PRIZE DRAWING

1:45 p.m.–BOTULISM IN CANNED FOODS
RALSTON B. READ

2:30 p.m.–ESCHERICHIA COLI IN FOODS
IRA J. MEHLMAN
A. C. SANDERS
N. T. SIMON
J. C. OLSON, JR.

2:50 p.m.–RECOVERY OF AFLATOXIGENIC MOLDS FROM FOODS IN THE HOME
G. S. TORREY
E. H. MARTH

3:15 p.m.–MILK BREAK
North Arcade

3:30 p.m.–SEAFOOD SANITATION AROUND THE WORLD
DON J. TOLADAY

4:15 p.m.–PREVENTING PROBLEMS IN THE FOOD INDUSTRY
ELMER H. MARTH

TUESDAY EVENING, AUGUST 13, 1974

7:30- 9:30 p.m.–EVENING DISCUSSION GROUPS

7:30 p.m.–FOOD SANITATION
Tampa Ray Room
RALSTON B. READ, *Moderator*
DONALD J. TOLADAY
DAVID CLEM
LOUIS A. KING

7:30 p.m.–MILK
Bay A & B
JOHN SPEER, *Moderator*
JOHN BRUHN
JOE ANTINK
DAVID BANDLER
WARD PETERSON
FLOYD BODYFELT

7:30 p.m.–ENVIRONMENTAL SANITATION
Coquina Key
DICK WHITEHEAD, *Moderator*
A. L. RIPPEN
DALE A. SEIBERLING
DOUGLAS J. VARNELL

WEDNESDAY, AUGUST 14, 1974

8:30 a.m.- 5:00 p.m.–REGISTRATION–Arcade
Message Center Cloakroom

MORNING—GENERAL SESSION
GRAND BALLROOM

EARL O. WRIGHT, *President*, Presiding

8:30 a.m.–DOOR PRIZE DRAWING

8:45 a.m.–FILM
"THE DAY MILK WAS TURNED OFF"
National Dairy Council

9:15 a.m.–AN APPROACH FOR ATTACKING WORLD FOOD PROBLEMS
WALTER F. WEDIN
Director of World Food Institute

10:00 a.m.–MILK BREAK
North Arcade

10:15 a.m.–DOOR PRIZE DRAWING

10:20 a.m.–ANNUAL BUSINESS MEETING
1. Report of Executive Secretary
2. Report of Secretary-Treasurer
3. Committee Reports
4. 3-A Symbol Council Reports
5. Report of Resolutions Committee
6. Report of Affiliate Council
7. Old Business
8. New Business
9. Election of Officers

AFTERNOON—MILK SANITATION SECTION
SOUTH BALLROOM

GUILLERMO GOMEZ, Presiding

1:30 p.m.–DOOR PRIZE DRAWING

1:45 p.m.–FILM
"MARVELOUS MILK MACHINE"
Dairy Farmers, Inc.
Orlando, Florida
JACK P. DODD

2:15 p.m.–BETALAINES AS COLORANTS IN DAIRY PRODUCTS
J. H. VON ELBE
J. H. PASCH
R. J. SELL

2:35 p.m.–SHELF-LIFE OF PASTEURIZED MILK STORED AT 4.5 AND 7.0 C.
S. V. PILKHANE
L. E. MULL
R. L. RICHTER
K. L. SMITH

3:00 p.m.–MILK BREAK
North Arcade

3:15 p.m.–"TEMPERATURE RECORDING – ITS EFFECTS ON QUALITY AND MARKETABILITY OF FLUID MILK"
LARRY CURTIS

4:00 p.m.–TEMPERATURE PROFILES OF PARISHABLE DAIRY PRODUCTS IN RETAIL FOOD STORES
FLOYD BODYFELT

AFTERNOON—
FOOD INDUSTRY SANITATION SECTION
BAY B

RON RICHTER, Presiding

1:45 p.m.–MICROBIOLOGICAL QUALITY OF GROUND BEEF AND CHICKEN PARTS FROM RETAIL STORE MEAT CASES
ROBERT J. HASIAK

2:30 p.m.–DEVELOPMENT OF AN IMPROVED METHOD FOR THE ISOLATION OF SALMONELLA FROM ACTIVE YEAST
W. H. ANDREWS
C. R. WILSON
P. L. POELMA

2:50 p.m.–CONTROL OF AFLATOXIN PRODUCTION IN WILD RICE
J. F. FRANK
G. S. TORREY
E. H. MARTH
D. A. STUIBER
R. C. LINDSAY
D. B. LUND

3:15 p.m.–MILK BREAK
North Arcade

3:30 p.m.–STATE SHELLFISH RECEIVING PROGRAM
DAVID CLEM

4:15 p.m.–PATULIN PRODUCTION IN CHERRIES BY PENICILLIUM AND ASPERGILLUS SPECIES
JOSEPH LOVETT
BRENDA BOUTIN
RUBIN G. THOMPSON

4:35 p.m.–INCIDENCE AND PROLIFERATION OF GOETRICHUM CANDIDUM IN REFRIGERATED CAKE YEAST AND FACTORS AFFECTING ITS GROWTH AND DETECTION
P. B. MISLIVEC
S. M. CICHOWICZ
V. R. BRUCE

AFTERNOON—
ENVIRONMENTAL SANITATION SECTION
TAMPA BAY ROOM

DOUGLAS J. VARNELL, Presiding

1:30 p.m.–DOOR PRIZE DRAWING

1:45 p.m.–THE PERFORMANCE OF PRESENT-DAY CIP SYSTEMS RELATED TO WATER AND CHEMICAL UTILIZATION
DALE A. SEIBERLING

2:30 p.m.–SOME ENVIRONMENTAL CULTURAL AND NUTRITIONAL FACTORS THAT AFFECT RUBRATOXIN FORMATION
C. O. EMEH
E. H. MARTH

2:50 p.m.–ENTERTOXIN RECOVERY IN FOODS UNDER CONDITIONS OF POLYELECTROLYTE EXCLUSION
R. W. BENNETT
D. L. ARCHER
W. T. AMOS

3:15 p.m.–MILK BREAK
North Arcade

3:30 p.m.–ENERGY CONSERVATION IN THE FOOD INDUSTRY
AL L. RIPPEN

4:15 p.m.–ASSESSING SAFE EXPOSURE LEVELS OF METHYL MERCURY
R. L. BRADLEY, JR.
A. G. HUGUNIN
W. E. RIBELIN

WEDNESDAY EVENING, AUGUST 14, 1974

6:00- 7:00 p.m.–RECEPTION
Lucayan Room

7:00 p.m.–ANNUAL AWARDS BANQUET
Grand Ballroom
EARL O. WRIGHT, *President*, Presiding
INVOCATION
IVAN E. PARKIN
INTRODUCTIONS
PRESENTATION OF AWARDS
ORLOWE M. OSTEN, *Chairman*

1. Past Presidents Award
2. Citation Award
3. Honorary Life Membership
4. C. B. Shogren Memorial Award
5. Sanitarians Award
   Sponsored by: Klenzade Products, Inc. Division Economics Laboratories, Pennwalt Chemical, Inc., Diversey Corporation, Inc.
6. Industry and/or Education Award
   Sponsored by Milking Machine Manufacturers Council, Farm and Industrial Equipment Institute

INSTALLATION OF OFFICERS
ENTERTAINMENT

THURSDAY, AUGUST 15, 1974

8:00 p.m.-12 Noon–Registration–Arcade
Message Center Cloakroom

7:30 a.m.–EXECUTIVE BOARD BREAKFAST MEETING
Coquina Key

NATIONAL MASTITIS COUNCIL 1974 REGIONAL MEETING

LUCAYAN ROOM

PROGRAM

RONALD RICHTER, Presiding

8:30 a.m.–GREETINGS FROM NMC PRESIDENT
DR. W. N. PHILPOT
THEME: MASTITIS CONTROL IN LARGE DAIRY HERDS

8:45 a.m.–FROM AN EXTENSION DAIRYMAN'S VIEWPOINT
D. M. SOLGER

9:15 a.m.–FROM AN EXTENSION VETERINARIAN'S VIEWPOINT
DR. G. W. MEYERHOLZ

9:45 a.m.–FROM A DAIRY FIELDMAN'S VIEWPOINT
KEN CROTHERS

10:15 a.m.–MILK BREAK
North Arcade

10:35 a.m.–FROM A LABORATORY PROCEDURE VIEWPOINT
ED CONNELL

11:00 a.m.–FROM A SANITARIAN'S VIEWPOINT
SAM O. NOLES

11:30 a.m.–FROM A DAIRY FARMERS VIEWPOINT
TOM CHRISTIAN

12:00 Noon–LUNCHEON BREAK

1:30 p.m.–J. A. ACREE, Presiding
IMPORTANCE OF PROPER MILKING MACHINE FUNCTION TO UDDER HEALTH MANAGEMENT
J. A. JARRETT

2:30 p.m.–IMPORTANCE OF MILK QUALITY: FROM THE COW TO THE CONSUMER
BOB SIMON

3:00 p.m.–MILK BREAK
North Arcade

3:20 p.m.–EXPERIENCES WITH THE GEORGIA MASTITIS PROGRAM
J. N. MADDUX

3:50 p.m.–NEW HORIZONS FOR NMC AND THE DAIRY FARMER
E. H. ROW

4:20 p.m.–QUESTIONS AND ANSWERS
(Panel with all Speakers)

5:00 p.m.–ADJOURN

5:15 p.m.–BOARD MEETING

ENTERTAINMENT

MEN AND WOMEN

SUNDAY, AUGUST 11, 1974

6:00- 7:00 p.m.–EARLY BIRD COCKTAIL PARTY
Hilton Room

MONDAY, AUGUST 12, 1974

6:00- 7:00 p.m.–RECEPTION
Lucayan Room

WEDNESDAY, AUGUST 14, 1974

6:00- 7:00 p.m.–RECEPTION
Lucayan Room

7:00 p.m.–BANQUET & ENTERTAINMENT
Grand Ballroom

THURSDAY, AUGUST 15, 1974

8:00 a.m.- 8:00 p.m.–DISNEY WORLD TRIP
Includes round trip transportation, Disney World admission plus admission to eight attractions

FRIDAY, AUGUST 16, 1974

8:00 a.m.- 8:00 p.m.–DISNEY WORLD TRIP
Includes round trip transportation, Disney World admission plus admission to eight attractions

ENTERTAINMENT FOR THE LADIES

(Ladies are invited to attend any of the meeting sessions)

TUESDAY, AUGUST 13, 1974

10:00 a.m.–TOUR OF ST. PETERSBURG AND SURROUNDING AREA
Lunch and Boat Ride on Bay

WEDNESDAY, AUGUST 14, 1974

10:00 a.m.–TOUR OF SUNKEN GARDENS
Luncheon and Fashion Show
Shopping Center Tour

LADIES HOSPITALITY ROOM

MONDAY-WEDNESDAY, AUGUST 12-14, 1974

9:00 a.m.- 5:00 p.m.–Hilton Room

PROGRAM PARTICIPANTS

DODD, JACK P., Director, Div. of Dairy Industries, Tallahassee, Fla.

EMEH, C. O., Dept. of Food Science, University of Wisconsin, Madison, Wisconsin

FRANK, J. F., Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

GOMEZ, GUILLERMO, McArthur Dairies, Ft. Lauderdale, Florida

HASIAK, ROBERT J., Food Science & Technology, Iowa State University, Ames, Iowa

HOTCHKISS, DONALD, Dept. of Statistics, Iowa State University, Ames, Iowa

HUGUNIN, A. G., Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

KING, LOUIS A., American Institute of Baking, Chicago, Ill.

LANE, C. BRONSON, Dairy Farmers, Inc., Orlando, Florida

LANE, JULIAN, State Senator, State Office Bldg., The Capitol, Tallahassee, Florida

LINDSAY, R. C., Dept. of Food Science, Univ of Wisconsin, Madison, Wisconsin

LOVETT, JOSEPH, Food & Drug Admin., Cincinnati, Ohio

LUND, D. B., Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

MARTH, ELMER H., Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

MEHLMAN, IRA J., Food Microiology Branch, Food & Drug Admin., Washington, D. C.

MISLIVEC, PHILLIP, B., Food & Drug Admin., Div. of Microbiology, Washington, D. C.

MULL, L. E., Univ. of Florida, Dept. of Dairy Science, Gainesville, Florida

OLSON, J. C. JR., Food & Drug Administration, Washington, D. C.

OSTEN, ORLOWE, M., Director, Dairy Industries Div., Minn. Dept. of Agriculture, St. Paul, Minn.

PARKIN, IVAN E., Retired, Westbrook, Conn.

PASCH, J. H., Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

PETERSON, WARD, Wisconsin Div. of Health, Madison, Wisconsin

PILKHANE, SHARAD V., Dairy Science Dept., University of Florida, Gainesville, Florida

POELMA, P. L., Food & Drug Administration, Washington, D. C.

READ, RALSTON B., Div. of Microbiology, Food & Drug Admin., Washington, D. C.

RIBELIN, W. E., Dept. of Veterinary Science, Univ. of Wisconsin, Madison, Wisconsin

RICHTER, RON L., Asst. Ext. Dairy Tech., University of Florida, Gainesville, Florida

RIPPEN, A. L., Extension Specialist in Food Science & Marketing, Michigan State University, East Lansing, Michigan

SANDERS, A. C., Food & Drug Administration, Washington, D. C.

SEIBERLING, DALE A., Asst. V. P., Equipment-Engineering Div., Economics Laboratories, Inc., Beloit, Wisconsin

SELL, R. J., Chr. Hansen's Laboratory Inc., Milwaukee, Wisc.

SIMON, N. T., Food & Drug Admin., Washington, D. C.

SKULBORSTAD, PARNELL J., V.P. Sales & Marketing, Babson Bros. Co., Oak Brook, Illinois

SMITH, K. L., Dept. of Dairy Science, University of Florida, Gainesville, Florida

SPECKMANN, ELWOOD W., Director of Nutritional Research, National Dairy Council, Chicago, Illinois

SPEER, JOHN, Milk Industry Foundation & Ice Cream Mfgrs. Assoc., Washington, D. C.

STUIBER, D. A., Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

THOMPSON, HAROLD E., U. S. Public Health Serv., Food & Drug Admin., Washington, D. C.

THOMPSON, RUBIN G., Food & Drug Administration, Cincinnati, Ohio

TOLADAY, DON J., V. P. Singleton Packing Corp., Tampa, Florida

TORREY, G. S., Dept. of Food Science, Univ. of Wisconsin, Madison Wisconsin

FLORIDA NEWS

An experiment to determine the best method of properly cleaning bulk milk transports that began in October of 1973, will be completed in a few weeks. Results will be presented at the IAMFES Annual Meeting in St. Petersburg.

At T. G. Lee Foods in Orlando six 6000 gallon milk transports of like construction were cleaned with both drop in and fixed spray ball devices. Temperature, time pressures, and solution strengths were monitored daily. Physical inspections were performed weekly of spray devices for the presence of foreign materials. The interiors of all tankers are inspected with both spot and black light and swab tests taken at six location in each tank weekly.

Additional information is being obtained from the recovery of pre rinse waters to utilize fats and milk solids normally lost. The use of inplace spray balls to air agitate milk and the cost of re-use verses single use wash solutions are two other studies that are included.

Taking an active part in the work by contributing equipment and supplies are Klenzade Products, Wyandotte Chemicals and Walker Stainless Steel. The Dairy Division of the Florida Department of Agriculture, the Florida State Board of Health's rating officers, and the Dairy Extension Service of the University of Florida are monitoring the study.

The results will be evaluated by the Dairy Science Department of the University of Florida and released nationally.

TRI-CLOVER DIVISION, LADISH CO., SELECTED FOR PRESIDENT'S "E" AWARD

(Left to Right) R. Nissen, Assistant Vice-President and General Sales Manager; R. Leitch, U. S. Department of Commerce; W. F. Gardner, Vice-President and General Manager; and W. Moore, Export Manager.

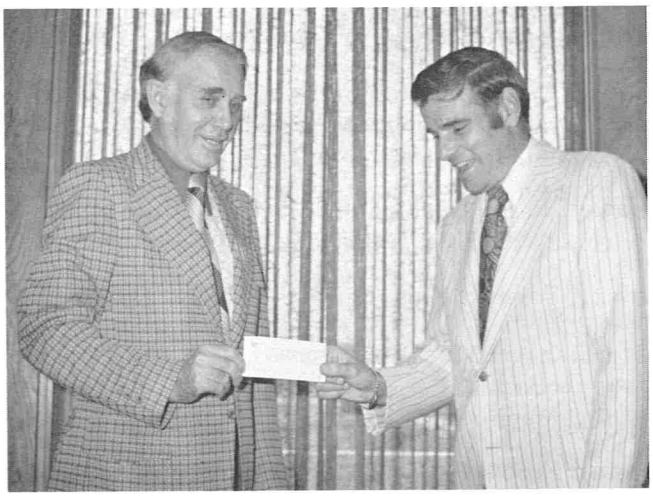
Secretary of Commerce Frederick B. Dent has selected Ladish Co., Tri-Clover Division, Kenosha, Wisconsin to receive the Presidential "E" Award for outstanding achievement in international trade.

The award was received by William F. Gardner, corporate vice-president, at a luncheon February 27, 1974, in Kenosha. It was presented by Russell H. Leitch, trade specialist in charge, Milwaukee District Office, U. S. Department of Commerce.

Following the luncheon, the "E" Award flag was raised at the Tri-Clover Plant, 9201 Wilmot Road, Kenosha.

The award was established by President Kennedy in 1961. Only 29 other firms in Wisconsin have won this highly coveted designation, and in the United States, only 1089 firms have been so recognized. Tri-Clover Division is the first firm in Wisconsin to receive the award this year.

EGG PRODUCERS JOIN DAIRYMEN IN NUTRITION PROGRAM



Dr. C. Bronson Lane, right, receives Florida Poultry Federation check from Barton Ahlstrom, FPF Executive Secretary, to further program of Dairy and Food Nutrition Council of Florida.

Florida's Dairy and Food Nutrition Council has received an important boost from state poultrymen which will allow further expansion of DAFNC's statewide nutrition education program, according to Dr. C. Bronson Lane, council executive director.

The Florida Poultry Federation is the latest food industry group to join with major state dairy cooperative members of Dairy Farmers, Inc. in support of the year-old nutrition council, Dr. Lane said. FPF, which is headquartered in Tampa, Fla., has made an initial allocation of $5,000 toward the council's 1974 program budget.

Barton Ahlstrom, executive secretary of the poul-

try group, said FPF members feel the well established nutrition council "is in a unique position to influence the consumption of eggs as well as dairy foods, while at the same time helping achieve better diets." Ahlstrom said the DAFNC program provides a "subtle sell" approach which takes full advantage of a revival of consumer concern for better nutrition.

Pointing out that stepped-up dairy advertising and nutrition education activities have helped increase milk consumption during the last couple of years, Ahlstrom cited that the egg industry would do well to intensify its promotional programs. He said that egg consumption has fallen from a per capita average of 400 to 297 in the past 15 years.

Ahlstrom said reasons for the decline parallel those in the dairy industry; including competition by substitute products, consumer confusion regarding nutritional values of foods, and continuing misinformation about the role cholesterol plays in the diet. The latter is of special concern to the egg industry because of the lingering, although unproven, contention that too many eggs in the diet may lead to atherosclerosis or coronary heart disease.

The egg industry will benefit from the council's program because DAFNC stresses the importance of a balanced diet, including milk, dairy products, and eggs, he said. "We look at eggs as complimentary, not competative, with milk and other dairy foods," he added, commenting that consumers traditionally link the two because of marketing and eating customs.

The DAFNC program is essentially aimed at "bridging the nutrition gap," explains Dr. Lane. He said the program includes training school teachers in nutrition education; with emphasis on the value of milk and milk products in the daily diet; clearing up misconceptions about proper diet through mass news media contact, and updating of health education leaders, physicians and dentists on the latest nutrition research findings.

He explained that two primary reasons the council emphasizes its teacher training program is because a large percentage of Florida's school children are on a sub-par diet; also experience has shown that efforts spent in conducting nutrition workshops for teachers are multiplied manyfold as the teachers transmit the information to many thousands of school (and pre-school) children throughout the state.

Dr. Lane pointed out that studies show that youngsters at the kindergarten and elementary grade levels have a high degree of retention, providing diet influence which carrys over into later life. Also, he said, it is apparent that when these youngsters are made aware of the value of milk (and eggs) in their diets at an early age, they grow up to be more responsive to industry advertising in later years.

Also important, he said, is the council's active information program aimed at mature audiences. Much of this is designed to offset misinformation on such matters as the diet-heart issue, and refute unwarranted claims that animal fat products contribute to heart attacks.

NATIONAL EXPOSITION FOR FOOD PROCESSORS AND CANNERS CONVENTION DRAW 7,000 TO ATLANTIC CITY

Some 7,000 persons attended the recent four-day National Exposition for Food Processors and National Canners Association convention here, reported W. D. Lewis, president, Food Processing Machinery and Supplies Association, which sponsors the exposition.

The 204 exhibitors were 50 more than in 1968, the last time the event was held here. Displays occupied 135,000 gross square feet and 75,000 net square feet in the East section of the giant Convention Hall.

Meeting highlights included an address by Secretary of Commerce Frederick P. Dent at the NCA All Industry Meeting and a variety of open sessions and workshops on such subjects as the energy crisis, water utility rules and consumer interests.

INDEX TO ADVERTISERS

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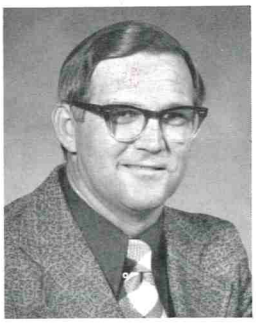
Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

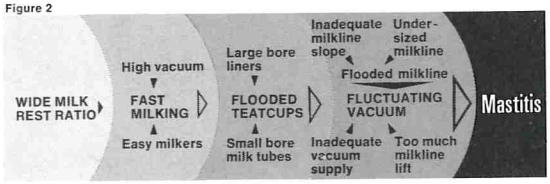
MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.

This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.