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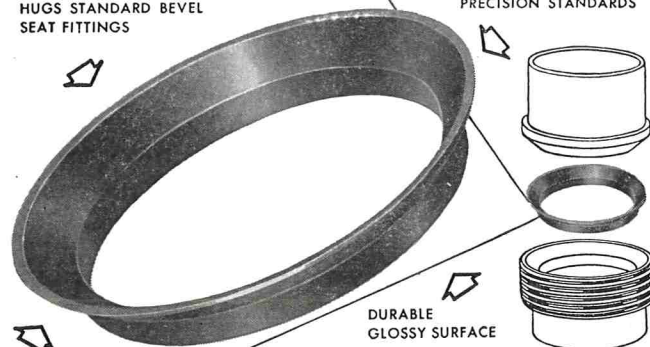
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METHODS FOR PRODUCTION OF HIGH QUALITY RAW MILK

(A Summary of Annual Reports Prepared From 1955 to 1970 by
the IAMFES Dairy Farm Methods Committee)

COMPILED AND EDITED BY

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

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THE ECOLOGY OF MILK PACKAGING

LESTER E. BARIBO
 Weyerhaeuser Company
 3400 Thirteenth S.W.
 Seattle, Washington 98134

(Received for publication August 30, 1971)

ABSTRACT

Man requires modern packaging to supply him with a clean and wholesome food supply. The relationship that the raw materials, processing, use, and disposal of milk packaging have with environmental factors is discussed. Which material or which packaging system has the least effect on these environmental factors depends on tradeoffs related to demographics, consumer habits and preferences, the unique interrelationships of environmental problems in specific communities and regions, and the way that legislation and regulations are developed and interpreted. But, in the final analysis, the problem is, how do we get each individual to take responsibility for his own individual pollution?

Ecology. A branch of science concerned with the interrelationship of organisms and their environment. The totality or pattern of relations between organisms and their environment.

What is the relationship between man, the materials he uses to package his food, and his environment? To determine the ecological effects of milk packaging, we must consider the total effects. These are raw materials, processing, distribution, and finally disposal. Within this concept must be man and how he manages, conserves, and utilizes these resources. How man manages these resources will affect other organisms, both for good and bad. No one system can be good for all. We must have a balanced system which provides the maximum utility to man and preserves the natural environment to the greatest possible extent.

Milk processing can be used as an example to show how different management systems affect the ecosystem. Raw milk normally will undergo a lactic fermentation. This results in what we call sour milk. Sour milk is prized by many cooks, and we deliberately sour milk to make cheese and fermented dairy foods. For health reasons and to increase the shelf life of the milk, it is pasteurized. This process kills the lactic organisms. The organisms that are left which finally spoil the milk cause bitter tastes and odors which make it undesirable for further consumption. As we can see, there are some benefits and some adverse effects.

MAN'S NEED FOR PACKAGING

It is apparent to everyone that a large amount of our resources are devoted to packaging. Why do we

need packaging? In the early days of our country most people obtained their milk from the family cow, or from the neighbor's family cow. It was most efficient to handle it in bulk in the bucket or pitcher. But as the population grew, the need developed for greater efficiency in production. Production became concentrated in certain areas. It then became necessary to divide the food products into smaller units that could be utilized by the consumer. It was necessary to protect food from contamination by disease organisms and from spoilage. For these reasons, modern packaging was developed. It is with packaging that we can supply the greatest number of people with a clean and wholesome food supply.

MILK PACKAGING STATISTICS

What is the magnitude of the milk packaging business? In 1968 (1), 27.2 billion quarts of fluid milk were sold. The first table shows the amount of milk packaged in each type of packaging system. These data show that paper dominates the milk packaging market; but glass also has a large stable market. The size of containers in which milk is packaged is shown in Table 2. The half-gallon size dominates the market.

The total number of each size of package is shown in Table 3. The half-pint size represents the largest number of milk packages.

The total raw material required for each packaging system is shown in Table 4. Glass and plastic con-

TABLE 1. FLUID MILK PACKAGE—1968

Glass	19.7%
Bulk	6.0
Plastic (One trip)	3.2
Plastic (Returnable)	0.5
Paper	70.6

TABLE 2. SIZE OF MILK PACKAGES

Gallon	18%
Half-gallon	52
Quarts	11
Pints	2
Half-pint	11
Bulk	6
Total	100%

TABLE 3. NUMBER OF CONTAINERS OF EACH SIZE (MILLIONS)

Gallon	1228.1
Half-gallon	7098.0
Quart	3003.0
Pint	1092.0
Half-pint	12012.0

TABLE 4. RAW MATERIAL REQUIRED FOR MILK PACKAGES (TONS)

Raw material	Based on % used in 1968	Based on 100% used in 1968
Glass	132,000 ^a	760,000 ^a
Plastic (One trip)	22,200	680,000
Plastic (Returnable)	1,000 ^a	135,000 ^a
Paper	600,000	860,000

^aBased on 20 recycles for glass and plastic

tainers require a cap, in addition to the basic material. The cap is generally made of paper. However, other materials such as plastic and aluminum foil are also used. In the left-hand column is listed the quantity of material required for each type of packaging based on use in 1968. In the second column is listed the amount that would be required if 100% of the milk in 1968 were packaged in each type of package. For the basis of these calculations, we have used an average recycle for the glass and plastic bottle of 20. Actual reports from the industry show the average recycle for glass is 19. We have not been able to get as reliable data for the plastic returnable bottle.

ECOLOGICAL EFFECTS OF PRODUCTION

The ecological impact of the milk package may be divided into three areas; production, use, and disposal. In production we must consider the raw materials and processing.

Raw material

Seventy-three percent of glass (4) is made from sand and limestone. Soda ash makes up most of the balance. These raw materials are abundant and do not produce a serious drain on our natural resources. From a conservationist point of view, mining of limestone and sand leaves unsightly blemishes on the earth's surface. The mining areas are fairly well confined, so it has little effect on wildlife. Limestone and soda ash production required for glass can contribute to air and water pollution. Large quantities of fine dust from the grinding of limestone usually permeate the air surrounding the plant. The Solvay process for soda ash requires limestone and common salt. The same problems arise here as in limestone production with the additional problem of water pollution from the residual calcium chloride. This is most frequently piled in huge dumps. With

rain it can be distributed into waterways. Approximately equal tonnage of calcium chloride waste by-product is produced for each ton of soda ash produced. For the percentage of soda ash used in glass, an equal percentage of waste products is produced from the raw materials.

Paper for milk packaging consists of approximately 90% cellulose and 10% polyethylene. Public health regulations require that the material be made from virgin fiber. An acre of old growth Northwest forest will yield about 160 tons of paper. Unaided, nature has required about 300 years for it to grow. An acre of Weyerhaeuser High Yield Forest will produce about 80 tons of paper and requires 40 to 60 years to grow.

Harvesting of wood for cellulose production can no longer be considered in the same class as gathering wild berries. Today's modern tree farms are as scientific an agricultural process as the growing of wheat or corn. The soil is prepared and the new forest is planted. It is fertilized and managed until ready to harvest. The only significant difference is that the growing period is 40 to 60 years rather than one year. In the old growth forests, no net growth of wood occurs, and little understory food is available for wild animals. The old forest only serves as an evening haven for these animals. The clear-cutting on tree farms opens up areas for food production. These areas remain very productive for wild animals' food for 10 to 20 years. With the rotation

TABLE 5. CALCULATION OF DISPOSABILITY RESISTANCE INDEX: 1966

Tonnage share material	Inciner- ation value	Sanitary landfill value	Open dumping value	Compost- ing value	Salvage value
Paper & paperboard	150	160	100	230	210
Glass	490	160	100	360	240
Plastics	300	270	100	480	330

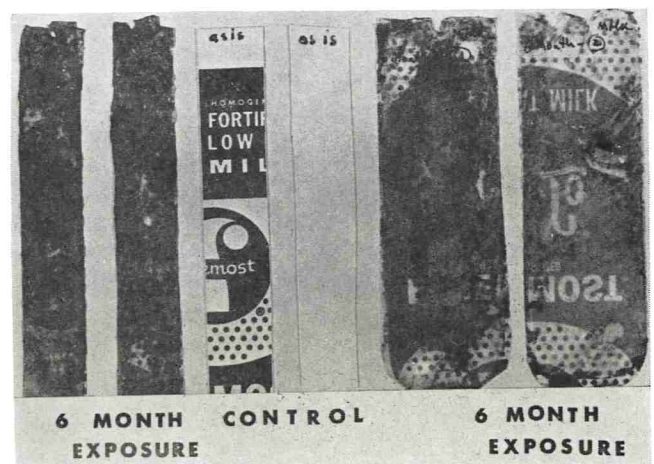


Figure 1. Sample of milk cartons in soil test for six months.

of cutting, new areas are constantly being opened providing a reliable food supply. Although clear-cutting is temporarily unsightly, a new crop of trees is planted within a year after harvest and will blanket the land with green within 5 to 10 years—renewing its beauty.

As our modern civilization increases its energy use, more and more carbon dioxide is discharged into the air. The new forest is an important balancing factor. One acre of Weyerhaeuser High Yield Forest growth will absorb approximately 7.4 tons of carbon dioxide and discharge 5.4 tons of oxygen per year. An average forest will absorb about 1.25 tons of carbon dioxide and discharge about a ton of oxygen per year. The need to maintain this balance with green plants cannot be overemphasized. An old forest can actually require more oxygen than it produces as a result of decay of waste materials.

A special area of concern is the amount of cull material which still must be left on the land. To manage the land, it must be disposed of. It is now burned to prevent forest fires and insect infestations, and to prepare the site for rapid reforestation. This may result in short-term local impacts on air quality. Weyerhaeuser Research is carrying out an intensive program to determine profitable ways to fully utilize these materials and energy sources.

Ethylene is the basic raw material of polyethylene. The processes generally used in this country are based on pyrolysis of petroleum fractions where ethylene is one of the fractions from the cracking operation.

The low molecular weight fractions are stripped from the natural gas supply. That which is not utilized is put back into the gas stream and used for home and commercial heating. The supply of ethane and propane exceeds the demand for polyethylene production so the ecological impact can be calculated on the basis of the weight of polyethylene produced and used.

Large spillage of oil on the soil from wells producing raw material for polyethylene can require many years to degrade. Since most degradation of crude oil is oxidative, conditions which deplete oxygen will slow the process. Transportation of oil on waterways creates a potential for water pollution problems.

Processes

The process for making glass (4) involves heating a mixture of sand and metal oxide to about 1,500 C. At this state, it flows sufficiently for forming. Often other materials are added. These decolorizing and refining agents aid in freeing the fused glass of undissolved gases.

The process yields little or no air or water pollution. A small quantity of waste slag must be dis-

posed of by solid waste disposal. The main factor is the large quantities of heat that are required to raise the mixture to 1500 C. This is calculated to be about 473,000 kilo calories per ton. It would require about 155 lb. of a typical coal to produce the heat. The burning of the coal would yield about 400 lb. of carbon dioxide and utilize about 290 lb. of oxygen per ton of glass.

All the paper for milk packaging made by the Weyerhaeuser Company is made by the kraft process. The raw material is chips, either from low grade logs or residual from lumber production. When chips are made from the above materials, about 30% of the material is left over in the form of bark, rotten fiber, and otherwise unusable parts of the log. These leftovers, which would otherwise become solid waste, are burned as a source of power for the operation. Hog fuel and spent liquor together furnish about 90% of the energy requirements for the process. The cooking liquor of the kraft process is made of sodium hydroxide and sodium sulfide. These chemicals are recovered from the spent liquor. So, from an ecological point of view, only the additional chemicals required per ton are of importance. Some 40 to 50 lb. per ton must be added to maintain the chemical level in the process.

Yields of pulp vary; but calculating a 50% yield, there is an equal quantity of spent liquor produced. In the kraft process these are primarily burned, recovering heat and chemicals. The water pollution potential resulting from one of our kraft mills is about 50 lb. of B.O.D. per ton of pulp. This can be reduced to about 12 lb. by the treatment process. There are also produced about 3 lb. per ton of suspended solids which must be disposed of by burning or land fill.

The air pollution problems of the kraft process are more complex. Sulfhydryl compounds are formed which create air quality problems. The human nose is more sensitive for these types of compounds than instruments that are available today. We can detect minute odors that are not precisely measurable. The actual quantity of material discharged is small. In a well closed kraft process this is about 0.024 lb. expressed as total reducible sulfur per ton and about 2 lb. of particulate matter. Yet, it is this small quantity which creates the main air problem in the kraft process. Using the basis of a 50% yield and 3 to 1 liquor ratio raised to 150 C, it is calculated that 810,000 kilo calories would be required to produce a ton of pulp. This would require 195 lb. of a typical coal. The burning of coal would require about 500 lb. of oxygen and produce about 700 lb. of carbon dioxide. As mentioned previously, about 90% of this fuel requirement is from the process itself and

hog fuel, rather than fossil fuel or other energy sources.

The ethylene for polyethylene production is separated from a mixture of gases produced in the cracking process. The process generally consists of high pressure and high temperature with a catalyst (3). Metal oxide is used, but other systems are also used. The heat of polymerization is reported to be about 25.4 kilo calories per mole of ethylene. This would require 910,000 kilo calories per ton of polyethylene produced.

It would therefore require the equivalent of 300 lb. of coal to make the conversion. This would require 720 lb. of oxygen and would discharge about 990 lb. of carbon dioxide per ton of polyethylene produced. Little or no solid waste is accumulated. The only significant water pollution problem known is the potential for thermal pollution. This results primarily from condensation waters used for cooling. This can have an impact on waters in which it is discharged. Air pollution is created by low molecular weight hydrocarbons. Many of these are burned, but the odor in the vicinity of such a plant can be a problem.

The calculation on oxygen consumption and carbon dioxide discharge reported here does not reflect the total for any of these processes. This would be extremely difficult to calculate even in our own operations. The calculations do attempt to show the relationship between the processes.

ECOLOGICAL EFFECT OF USE

The way a milk package is used can have an effect on the ecology. In this area we must consider the single service container vs. the returnable container.

There has been some agitation by some groups based on a so-called ecology purpose, to recommend that we use returnable bottles. Aside from the fact that most consumers seem to prefer a non-returnable bottle, there are other facts which affect the ecology. The returnable bottle must be cleaned. The returnable bottle may be used for many purposes besides the storage of milk before it is returned to the dairy. The impervious nature of glass permits it to be cleaned and sanitized at the dairy. The same cannot be said for the plastic bottle. The plastic bottle will absorb some materials which cannot be removed by cleaning. Dairies using the plastic returnable bottle use a sniffer which detects organic solvents. It rejects those bottles in which it detects these materials. However, it cannot detect many other possible materials that may have been stored in the bottles.

One hundred percent use of returnable glass bottles would decrease the solid waste disposal of milk pack-

aging based only on the package by 12% over paper. Similarly, the single service plastic bottle reduces the solid weight disposal by 26% over paper. Approximately 10% of the solid waste disposal of the glass and plastic would be represented by the cap. The cap would have a disposability the same as a paper carton. The balance would have increased disposal problems. An additional problem of a returnable bottle is the detergents required for cleaning. Based on a study at Michigan State University (5), we have estimated the detergents required to clean the returnable bottles if they were used exclusively to be about 10,000 tons. It would require 2,400 million gallons of water per year. Since most dairy industry detergents consist largely of phosphates, this would be a water pollution problem where the receiving waters are phosphorous limited. Calculating on the basis that 70% of the detergent is phosphate and that the federal government places a limit of 0.5 ppm of phosphate in water before eutrophication starts, the amount of phosphate in the detergent would require a lake one-fourth the size of Lake Erie to dispose of the phosphate without damaging consequences to the ecosystem.

Additional transportation is required for the returnable bottle. This includes transportation for returning the bottle to the dairy as well as the increased weight of the glass bottle.

DISPOSAL

Having been produced, the package must now be disposed of. The Midwest Research Institute (2) developed an index for disposability considering each disposal method. In Table 5 there is a value of disposability for each type of packaging material with a different type of disposal. A value of 100, which is the lowest value, indicates a very good disposability by the particular disposal method in question.

A value of 500 is the highest value and indicates very poor disposability. Hence, paper, easily disposable through incineration, is rated 150. Glass, which is not combustible, has a very high figure of 490.

Plastic does not burn readily and can give off large quantities of smoke. It tends to melt and create problems in the incinerator. Because of the very thin film of polyethylene on the paper carton, the cellulose furnishes enough energy to burn the plastic readily without smoke. As we go to more efficient incineration methods in which the plastic will oxidize, any intermixed glass waste becomes fluid and creates problems.

In land fill, glass and paper are equal at 160. The paper will decompose and the glass can be broken to provide inert fill material. Plastic will neither

decompose nor break. This creates voids, thus creating problems in the land fill. The poorer 270 rating results.

Open dumping cannot be considered a satisfactory method of disposal. Composting would be an excellent way to dispose of organic material if the value of the material would pay for the cost.

Our own laboratory studies have shown that 85% to 90% of paper milk cartons are biodegradable in 6 months' time. Figure 1 shows a picture of some samples after 6 months in soil test.

Salvage and reuse is being touted as the most acceptable means of disposal. The Glass Container Manufacturers Institute has announced a nationwide program for buying back bottles for use in making new bottles. A relatively small percentage of plastics is reused. Plastic materials are highly vulnerable to contamination. They do not lend themselves to reuse. Dow, however, has reported a program to make plastic tile from used plastic milk containers.

In 1966 about 20% of the fiber used in paper and paperboard came from reclaimed paper. This is usually corrugated boxes, newspaper, or other paper materials that are available in large uniform supply. It does not include significant quantities of packaging materials, particularly milk cartons. Therefore, if we are to consider recycling of milk cartons, we must develop better alternative end products. Our company does have a mill in the Midwest which uses waste paper as its fiber source. Other mills use waste paper from our own operations where control of supply and quality are maintained.

There are two objectives in disposal: (a) to conserve natural resources, and (b) to ultimately dispose so that it no longer has an effect on the environment. Metals do lend themselves to recycling. And for metals at least, I would consider recycling essential in order to conserve this natural resource. Glass lends itself to ultimate disposal in building material, roads, etc., and also to recycling. However, the need to conserve this natural resource does not seem to apply as there are vast quantities of raw materials available. Plastic and paper do not lend themselves too well to recycling. Reuse of these materials results in lower and lower quality. This does not mean that they should not be recycled during their useful life, but the pipeline will soon become full and a method of ultimate disposal will be needed. Incineration for power production appears to me to be the best ultimate use for these materials. The raw material for paper is a renewable resource and an increasing supply should be available if man manages this resource properly. The raw material of

plastic is not renewable and conservation of its use should be practiced.

SUMMARY

Having reviewed many of the factors which are related to the ecology of milk packaging, what conclusion can be come to? Certainly not all is black or white. How do you balance the different factors with different materials as it affects the ecosystem? There obviously is no single answer. Tradeoffs relate to demographics, consumer habits and preferences, the unique interrelationship of environmental problems in specific communities and regions, and the ways that legislation and regulations are developed and interpreted. However, I do believe that the marketplace is a good arbitrator. The government has a responsibility to determine the limits of tolerance within which an industry may alter the balance of nature. Some positive steps have been taken. Very strict and positive air and water pollution control is now being required of the paper industry. These factors will affect the cost or market price. This will affect their final use as the packaging material of choice by the consumer. The cost of disposal is going to become an important factor in these costs in the future. The impact of nonrenewable resources will be felt more as the government applies increasing pressure to conserve these resources.

I do feel that each of us as individuals and as companies are going to have to take on a greater individual responsibility for the disposal of materials that we use and produce. Milk, which is consumed to a large degree by the young in our population, may be used as a vehicle to bring awareness of the problem to the next generation. Educational programs should be started, both at the company level and in our schools. But, in the final analysis, the problem is—how do we get each individual to take responsibility for his own individual pollution?

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EFFECTS OF TIME OF HOLDING DILUTIONS ON COUNTS OF BACTERIA FROM RAW MILK¹

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ABSTRACT

Raw milk samples were diluted with buffered water and held at room temperature for periods up to 20 min before plating. There was an increase in counts at the 95% but not at the 99% level of significance. Most of this increase appeared at 10 min holding time. Interaction effects were highly significant ($p < 0.01$) between holding times and investigators and also between treatments and samples within investigators. It is suggested that the holding time of dilutions to be used for the standard plate count be no longer than 5 min.

The antibacterial effects of sea water are well known (3, 11) and probably account for the rapid disappearance from it of bacteria such as the typhoid bacillus. Less work has been done on the survival of bacteria in demineralized water although Carlucci and Pramer (3) indicated that in their studies, *Escherichia coli* died more rapidly in demineralized water than in water containing 25% sea water. Butterfield (2) studied recovery of bacteria from river waters after 15 and 30 min in various dilution fluids and observed a diminution in counts. Better survival of bacteria was observed in dilute phosphate buffer or

phosphate buffer fortified with calcium chloride, magnesium sulfate, and ferric chloride. This work was probably the origin of the recommendation by *Standard Methods* for incorporating dilute phosphate buffer in the dilution fluid for the plate count. The amount of phosphate buffer recommended by Butterfield is the same as that suggested by *Standard Methods*.

The effect of the dilution fluid on bacteria of raw milk has not been investigated to any extent. *Standard Methods* (1) recommends that not more than 20 min elapse from the time that the milk sample is diluted to the time it is plated. Geldreich and Clark (5) devised a test for determining the suitability of distilled water for microbiological use based on the growth and survival of *Aerobacter aerogenes*. They found that some water samples supported growth of *A. aerogenes* after a 24 hr incubation period; others were either toxic (due to chlorine) or had no effect. Garvie (4) found that *E. coli* and *Pseudomonas fluorescens* would grow in distilled water when nutrients or buffer were added. Price and Gore (13) found certain distilled waters to cause erratic results in folic acid assays with *Streptococcus faecalis* R and postulated the existence of volatile inhibitors, other than chlorine, in certain distilled waters.

The growth rates at different temperatures of the predominant bacteria of raw milk, the psychrotrophs, have been studied by Jezeski and Olson (10), Huhtanen (8), Greene and Jezeski (6), Heather and Vanderzant (7), Lawton and Nelson (12), and others. In all instances, the psychrotrophs grew readily at near-room temperatures (20-30 C). The present study was undertaken to determine the effect of short periods of holding diluted raw milk on recovery of bacteria from it.

MATERIALS AND METHODS

Methods advocated by *Standard Methods* (1) were followed for the plate counts except when counts of <30 per plate were encountered. In this instance statistical procedures forced us to use the actual numbers. The protocol for the experiment and the statistical analyses were in general similar to those of a previous study (9). Since there were significant interaction effects between treatments and investigators and between treatments and samples within investigators, the standard model for expected mean square E (MS) for the

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mixed model, as in this experiment, did not include a satisfactory denominator for determining significance of the F statistic. A quasi F ratio (Satterthwaite's correction) was derived using a denominator for mean square and degrees of freedom as outlined by Winer (14). The Hartley test for inhomogeneity of variances was used as outlined by Winer (14).

Results from ten investigators were included in the study. The results of investigator D were composed of two analysts, D₁ and D₂, each of whom assayed different milk samples. Five dilution bottles containing 99 ml of phosphate buffered water (either demineralized or distilled depending on the normal supply) were inoculated with 1 ml of raw milk obtained either from farm bulk tanks or from holding tanks at processing plants. The dilution bottles were mixed by gently inverting and were held at room temperature at specified times. One bottle was shaken according to *Standard Methods* recommendations and plated in duplicate as soon as possible, using 1 or 0.1 ml of the dilution fluid. Plate counts used for the analyses were, for each milk sample, from either the 1-100 or 1-1000 dilution even though the colony counts might have been <30 or >300. Results were transformed into log₁₀ for the analysis-of-variance and were calculated using an IBM 1130 computer.

RESULTS AND DISCUSSION

Average plate counts

Table 1 shows the plate counts obtained by the different analysts at the five different holding times of 0, 5, 10, 15, and 20 min. These counts were from either the 1-100 or 1-1000 dilution but for convenience were transformed in the table to 1-1000 equivalent counts. The overall average counts were highest with a 10-min holding time and were about 28% more than the control which was shaken and plated immediately. Counts were higher than controls at all holding times; although the increase was only 4.1% at 5 min. Six investigators (B, C, D₁, F, H and I) found increases in counts at all holding times; two (D₂ and G) found decreases at all holding times. Investigator A found decreases at all holding times except for a slight increase at 10 min. Investigator E found increases at all holding times except 5 min when there was a decrease.

Statistical evaluation

The analysis-of-variance for this experiment is shown in Table 2. The degrees of freedom for the denominator for the F test of treatment effects were calculated to be 24 by the method of Winer (14). The denominator mean square was calculated to be 0.0151 using the Satterthwaite formula.

There were highly significant differences between investigators and, as expected, between samples. The holding time effects were of significance with $p < 0.05$ but not with $p < 0.01$. Some of the reasons for this low level of significance were the highly significant interaction effects encountered (lines d and e). Such interactions were found before (9) and may represent investigator bias, differences in types of

bacterial flora, variable storage times of the bulk milk, etc.

Further analysis of the treatment effects showed a highly significant linear but an insignificant quadratic trend even though the means from Table 1 indicated that counts were highest at 10 min and then fell at 15 min rising again at 20 min.

The treatments times investigators interaction was highly significant. Part of this interaction resulted from differences in regression lines among the investigators (non-parallel lines); however, another substantial portion of this interaction was unexplained. The slopes of the regression lines for investigators varied from 0.000038 for investigator A to 0.187180 for investigator I; there were obviously great differences in the way the different milk samples behaved when held for different times in the dilution bottles. This could be a reflection of the types of bacteria present or their stage in the growth cycle.

Tests for reproducibility

Single-degree-of-freedom variances were calculated for each pair of observations and were summarized as shown in Table 3. The Hartley test for inhomogeneity of variances [Winer (14)] was made using the statistic

$$F_{\max} = \frac{\text{largest of } k \text{ variances}}{\text{smallest of } k \text{ variances}} = \frac{0.006053}{0.000927} = 6.5$$

with $k = 10$ (number of investigators) and $M-1$ degrees of freedom (29) for each investigator. The F_{\max} of the Hartley test for inhomogeneity of variances exceeded the tabled F_{\max} statistic at the 99% level of significance of approximately 3.4. Although the hypothesis of equality of variances was, therefore, rejected, it was felt that the analysis-of-variance test was still robust enough to withstand these inhomogeneities. Alternatively, one could eliminate the "outlying" variances and calculate the analysis-of-variance with the more homogeneous deviations; this, however, would have required a *post facto* decision and its justifiability could be questioned.

Another test for determining inhomogeneity of variances was made using an analysis of the variances obtained from investigators and holding times (Table 4). The variances of Table 3 were transformed to log₁₀ and a two-way analysis of variance was done. There were no significant differences between the variances of holding times but there was a highly significant difference between investigators ($p < 0.01$). The variance of investigator B was lower than the others—an effect also observed previously (9). A test of this variance against the others was made using an orthogonal contrast—the obtained F ratio was 22.0 and indicated a highly significant ($p < 0.01$) decrease in variance (increased reproducibility between

TABLE 1. EFFECT OF DILUTION BOTTLE HOLDING TIME ON PLATE COUNT

Investigator	Milk sample no.	Holding time (min)									
		0		5		10		15		20	
A	1	4.5*	5.2	4.5	4.9	5.3	3.8	4.3	4.9	4.3	4.9
	2	4.2	6.2	6.2	5.1	6.9	7.8	6.6	8.8	6.6	5.8
	3	6.5	5.6	5.0	4.4	5.1	5.2	4.3	5.0	5.6	5.3
	4	9.9	10.0	6.9	8.6	10.5	7.8	7.6	9.8	9.0	10.0
	5	4.4	6.1	5.0	4.2	5.4	5.9	5.5	4.1	5.3	4.7
	6	22.7	24.9	26.5	21.4	21.9	25.8	22.1	20.4	22.9	21.6
	Average		9.18		8.56(-)		9.28(+)		8.62(-)		8.83(-)
B	7	7.0	7.2	7.7	7.0	7.5	7.8	7.9	7.6	8.1	8.7
	8	2.9	3.2	3.9	4.1	3.3	4.0	3.4	4.1	4.8	4.2
	9	110	120	108	121	116	127	118	134	115	129
	10	62.0	58.0	64.0	59.0	67.0	63.0	69.0	82.0	82.0	75.0
	11	4.5	4.8	4.7	5.3	5.8	5.5	6.1	6.4	6.1	6.0
	12	24.0	23.6	26.6	24.7	27.5	25.0	29.6	25.5	28.3	26.7
	Average		35.6		36.3(+)		38.3(+)		41.1(+)		41.2(+)
C	13	111	119	132	116	148	118	159	132	100	159
	14	980	1000	850	980	1200	1300	1200	1000	1300	1200
	15	228	286	366	388	490	586	414	534	516	416
	16	9.2	12.0	9.2	8.4	8.4	8.8	10.4	8.2	9.3	11.2
	17	340	380	350	370	780	520	390	444	350	464
	Average		346.5		357.0(+)		515.9(+)		429.2(+)		452.6(+)
D ₁	18	11.9	13.6	14.0	12.4	13.2	15.0	12.2	14.0	11.0	12.5
	19	9.0	9.0	7.6	8.5	8.3	12.7	10.4	9.0	8.4	13.0
	20	196	226	227	238	252	244	316	278	256	296
	21	7.8	7.1	7.9	7.5	8.0	5.5	7.4	9.5	6.1	12.0
	22	13.7	15.7	13.7	15.0	14.1	15.2	16.7	14.9	13.6	16.6
	23	9.7	9.5	9.0	7.8	10.0	9.7	7.7	7.3	10.7	3.6
	24	86.0	77.0	73.0	60.0	69.0	75.0	67.0	70.0	75.0	69.0
	Average		49.4		50.1(+)		53.7(+)		60.0(+)		57.8(+)
D ₂	25	5.6	6.0	7.3	7.1	6.4	8.5	5.9	9.5	7.3	7.2
	26	12.5	12.0	11.5	14.5	10.0	12.0	13.0	12.9	12.1	13.5
	27	8.2	6.7	7.7	9.0	7.2	9.8	8.8	8.9	7.7	6.4
	28	43.0	41.0	39.0	35.0	21.0	32.0	42.0	30.0	31.0	45.0
	29	23.5	21.5	18.5	18.5	22.6	21.4	21.5	19.6	24.5	23.0
	30	4.5	4.7	4.4	4.4	5.1	4.1	4.1	4.3	4.3	5.1
	Average		15.8		14.7(-)		13.3(-)		15.0(-)		15.6(-)
E	31	13.4	11.0	12.5	11.8	11.5	11.4	13.3	12.5	11.1	13.7
	32	6.0	5.4	7.4	6.7	8.1	6.8	8.7	10.2	10.2	12.1
	33	7.1	6.8	7.2	5.0	7.2	5.5	6.4	5.0	6.1	6.2
	34	85.0	96.0	77.0	90.0	90.0	103.0	94.0	97.0	95.0	90.0
	35	3.4	2.9	3.3	3.2	3.4	3.0	3.8	3.8	3.4	3.5
	36	133	147	124	142	137	146	136	146	147	160
	Average		43.1		40.8(-)		44.4(+)		44.7(+)		46.5(+)
F	37	22.7	22.5	21.6	23.0	21.8	22.1	23.5	25.6	22.9	23.5
	38	8.7	10.0	9.8	9.0	9.4	9.2	12.1	8.9	11.2	9.3
	39	7.9	9.4	8.1	7.9	9.4	8.8	10.4	9.6	8.1	6.9
	40	3.8	3.4	5.2	3.8	4.2	4.9	2.9	4.5	2.3	3.9
	41	1.3	1.6	2.5	1.7	1.8	1.7	1.6	1.2	1.3	1.6
	42	5.6	5.6	6.7	8.6	6.0	6.4	9.3	7.5	5.5	5.9
	Average		8.54		8.99(+)		8.81(+)		9.76(+)		8.58(+)
G	43	131	222	170	162	139	134	117	142	163	152
	44	13.1	11.2	10.0	13.1	13.6	11.5	12.3	11.3	11.8	11.9
	45	8.3	7.1	9.0	8.9	6.4	11.9	5.2	5.4	6.2	8.4
	46	1.9	1.8	3.2	2.0	0.9	0.8	1.0	1.0	1.9	1.3
	47	49.0	40.0	54.0	33.0	40.0	53.0	50.0	43.0	36.0	51.0
	48	6.2	6.7	6.1	7.5	8.7	7.7	6.2	6.6	6.7	7.4
Average		44.3		39.9(-)		35.6(-)		33.4(-)		38.1(-)	

H	49	5.9	3.3	4.5	4.3	4.4	4.9	5.6	6.5	5.8	5.3
	50	7.3	6.2	6.4	6.7	8.8	10.2	7.4	7.6	7.1	6.0
	51	6.7	6.9	5.4	7.4	6.3	5.0	5.1	5.0	5.4	6.9
	52	10.9	12.3	12.1	11.4	12.0	13.9	23.8	21.8	13.4	14.0
	53	4.0	4.0	4.2	6.2	2.5	4.1	3.2	2.4	5.7	4.5
	54	39.0	38.0	54.0	47.0	84.0	91.0	75.0	54.0	48.0	33.0
	55	10.1	10.4	13.4	14.0	11.3	14.4	14.2	15.1	16.0	18.2
	56	13.3	13.0	14.7	15.6	13.2	13.7	14.7	13.0	11.5	11.4
Average		12.0		14.2(+)		18.7(+)		17.2(+)		13.3(+)	
I	57	65.0	35.0	77.0	68.0	126	98.0	123	150	118	152
	58	23.5	22.5	26.8	29.0	26.0	25.7	21.9	25.0	27.3	32.0
	59	125	123	165	128	165	190	155	160	160	163
	60	81.0	78.0	108.0	97.0	155	95.0	188	105	210	112
	61	87.0	86.0	95.0	140	110	102	117	100	108	105
	62	23.6	26.2	24.5	27.9	26.7	25.0	27.6	26.4	24.2	24.7
	63	21.0	22.2	20.6	22.3	20.1	21.9	25.6	23.5	21.7	24.9
Average		58.5		73.5(+)		84.7(+)		89.1(+)		91.6(+)	
Overall Average		56.05		58.36		71.60		65.69		68.19	
Increase %		—		4.1		27.7		17.2		21.6	

*For actual counts multiply by 1000. (+) is increase in counts from 0 time; (—) is a decrease.

TABLE 2. STATISTICAL EVALUATION OF PLATE COUNTS

Line	Source of variation	Degrees of freedom	Sum of squares	Mean	F ratio ¹	Significant with	
						p < 0.01	p < 0.05
a	Investigators	9	99.7044	11.0782	4.07	yes	yes
b	Samples within investigators	53	144.2401	2.7215	878	yes	yes
c	Treatments	4	0.1697	0.0424	2.81	no	yes
	linear trend	1	0.1466	0.1466	9.71	yes	yes
	quadratic trend	1	0.0208	0.0208	1.38	no	no
	cubic trend	1	0.0009	0.0009	<1.0	no	no
d	quartic trend	1	0.0611	0.0011	<1.0	no	no
	Treatments times investigators	36	0.6535	0.0182	2.22	yes	yes
	non-parallel lines	9	0.2398	0.0266	3.25	yes	yes
e	residue	27	0.4137	0.0153	1.87	yes	yes
	Treatments times samples within investigators	212	1.7345	0.0082	2.64	yes	yes
f	Error (between duplicate plates)	315	0.9866	0.0031	—	—	—
TOTAL		629	247.4888				

¹F ratios were lines a/b, b/f, d/e, e/f. F ratio for treatment effects included a denominator mean square of 0.0151 derived from Satterthwaite's correction with 24 degrees of freedom.

TABLE 3. AVERAGE VARIANCE ESTIMATES

Investigator	Holding time (min)					Average variance
	0	5	10	15	20	
A	0.004867	0.002917	0.003900	0.004383	0.001017	0.003430
B	0.000417	0.000800	0.000967	0.001667	0.000750	0.000927
C	0.002620	0.000960	0.004820	0.003880	0.007200	0.003904
D ₁	0.001043	0.001328	0.004743	0.001828	0.010500	0.003897
D ₂	0.000917	0.001417	0.006850	0.005517	0.003417	0.003633
E	0.001600	0.002983	0.002217	0.001500	0.001317	0.001927
F	0.001650	0.005033	0.000567	0.006750	0.003433	0.003493
G	0.005917	0.009133	0.008200	0.001133	0.005850	0.006053
H	0.004500	0.003338	0.004938	0.002825	0.003662	0.003858
I	0.005400	0.003643	0.004571	0.005800	0.006800	0.005248
Average variance	0.002893	0.003155	0.004177	0.003528	0.004395	0.003637

TABLE 4. ANALYSIS OF VARIANCE SUMMARY OF VARIANCE ESTIMATES FOR INVESTIGATORS AND HOLDING TIMES

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	Significant with	
					$p < 0.01$	$p < 0.05$
Investigators	9	2.4169	0.2685	3.63	yes	yes
Treatments (holding times)	4	6.4243	0.1060	1.43	no	no
Investigators times treatments	36	2.6617	0.0739			
TOTAL	49	5.5029				

duplicate plates) as compared to the nine other investigators.

It is rather interesting that the counts increased so remarkably in only 10 min in the dilution bottles. The shock of dilution might have been expected to cause a decrease in counts. The increase may have been a result of growth of the organisms or may have been caused by a breaking-up of clumps of bacteria. It might be argued that if the clumps of bacteria were indeed broken up, resulting in a more homogeneous suspension, then the counts should have shown a greater reproducibility with time of holding. Tables 3 and 4 did not indicate such a difference.

The 12th edition of *Standard Methods* (1) specifies that not more than 20 min elapse between diluting and pouring of the plates. According to the work reported here such an indeterminate interval would lead to widely disparate results. For instance, the counts after 5 min would be 4% higher; after 10 min they would be 28% higher; and at 20 min they would be 22% higher. The closest approximation to the true counts would seem to be a 5-min holding time. We would suggest that this time interval be considered as the maximum time allowed between diluting and pouring of the plates.

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FLAVOR EVALUATION OF VARIOUS MILK, VEGETABLE, AND MARINE PROTEIN SOURCES¹

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ABSTRACT

Sensory and analytical measurements were made on eight milk and six vegetable and marine protein samples. Sensory panel scores for blandness were recorded for odors of dry products and for odor and taste of 3% suspensions in water. Gross composition, GLC headspace vapor analysis, and carbonyl level were obtained. Odor of cottonseed flour powder was judged the most bland, whereas reconstituted low heat NFDM rated most bland in both odor and taste. The least bland product was fish protein concentrate. Moisture and protein ranged from 2.1 to 9.6 and 11.8 to 92.0%, respectively. All milk products had lower total GLC vapor peak areas than the vegetable proteins. Carbonyls varied from 2 mg/kg in sodium and calcium caseinate to 220 mg/kg in toasted defatted soy flour. In most instances, total GLC vapor areas of carbonyl levels did not agree with sensory panel data.

The potential for protein supplementation of human foods has been well documented (9, 11). Numerous milk-based protein supplements have been available for quite some time and have found widespread acceptability. However, recently considerable attention has been given to the use of vegetable and marine protein sources.

One of the major assets of milk protein materials has been their relatively bland flavor whereas the major limitation with most non-milk protein supplements has been their unfamiliar or objectionable flavor characteristics (2, 8, 16). As a result, most non-milk protein based products find their greatest utilization in animal feeding although they represent a good source of protein for humans.

This study was undertaken to compare both sensory and certain chemical properties of presently available representative protein supplements. It was hoped that the study would demonstrate which supplements meet the flavor acceptability standards of today's consuming public and point out specific flavor deficiencies in others:

MATERIALS AND METHODS

Products

A total of 14 recently obtained commercial protein supplements were evaluated. These included eight milk-based products: low and high heat non fat dry milk (NFDM), whole

milk powder, non-demineralized and demineralized whey powder, buttermilk powder, and sodium and calcium caseinate. Soy products included isolated soy proteinate, soy protein concentrate and toasted, defatted soy flour. Toasted soy flour was used because of the report that improvement in flavor resulted from toasting (16). The remaining supplements included peanut flour, fish protein concentrate, and cottonseed flour. Samples were refrigerated until analyzed.

These studies represent findings from single samples from a limited number of manufacturers and thus may not be completely representative of all similar products on the market.

Approximate analysis

All samples were analyzed for moisture, protein, fat, ash, and fiber (3). In addition, the pH values of 3% distilled and demineralized water suspensions were taken.

Sensory panel

Twenty college-age female students participated in the odor and taste evaluations. The panel was instructed to evaluate the room temperature samples for blandness only (defined as completely free of a detectable odor or taste) with a score of 10 indicating a "completely bland" response and a score of 1 representing a "strong" impression. Random samples were evaluated for odor intensity in the powder form by sniffing three times and rating the blandness of 5 g of powder in a 35 ml capacity screw cap vial. The odor intensity and flavor of 3% samples reconstituted in distilled and demineralized water were evaluated by placing 20 ml of liquid sample in a thoroughly cleaned and odor-free 50-ml beaker and asking the panel to first sniff and then taste the samples. When tasting, the panel was reminded to separate odor from flavor and to judge blandness by their flavor impressions only. The liquid samples were reconstituted 0.5 hr before pouring into beakers for evaluation. All coded samples were presented to the panel on three separate occasions over a 2-week period. All sampling was done in a quiet and odor-free room and panelists were instructed to ignore any differences in color and particle size among samples.

Headspace analysis

Direct headspace vapor samples were prepared in a manner similar to the procedure of Bassette and Ward (4). The 6.5-ml serum vials contained 1.2 g anhydrous sodium sulfate, 2 ml distilled, demineralized water, and 0.5 g of sample powder. The mixture was heated for 10 min in a 60 C waterbath and 1.5 ml of vapor was withdrawn for sample injection.

A Hewlett-Packard model 5750 dual hydrogen flame gas chromatographic unit was used. The 2.5 m column was packed with 20% Carbowax 20 M on 60-80 mesh HMDS Chromosorb P (Applied Science). All samples were evaluated at an oven temperature of 85 C isothermal with a nitrogen flow of 65 ml per minute. Total GLC peak areas were calculated by measuring peak height by width at one-half height for each peak.

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Carbonyl content

The method of Lappin and Clark (14) was used to determine the carbonyl content of all samples.

Statistical analysis

The sensory data were submitted to a form of confidence interval evaluation developed by Tukey (19). All data were treated at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Chemical composition

The ranges in gross chemical composition can be seen in Table 1. Most of the products had moisture values below 5%. However, some of the vegetable protein products contained > 6% and cottonseed flour approached 10% moisture. These latter products could be susceptible to browning reactions upon storage (13) which might result in unacceptable color and flavor characteristics.

Protein levels ranged from 11 to 92% on an "as is" basis. The caseinates and soy proteinate represented the most concentrated source of protein, whereas several milk by-products contained the lower protein values.

Residual lipid material ranged from < 0.5% in

TABLE 1. APPROXIMATE ANALYSES¹ OF MILK, VEGETABLE AND MARINE PRODUCTS EVALUATED

Product	Moisture (%)	Protein (%) ¹	Fat (%)	Ash (%)	Fiber (%)	pH ²
Low heat NFDM	3.2	35.7	1.0	8.1	—	6.79
High heat NFDM	3.1	35.6	1.0	8.1	—	6.85
Whole milk powder	2.1	26.3	27.6	5.9	—	6.83
Whey powder	4.7	11.8	1.1	8.1	—	6.06
Demineralized whey powder	4.0	35.2	3.1	2.8	—	6.65
Buttermilk powder	4.3	34.1	5.2	7.5	—	6.65
Sodium caseinate	3.6	90.3	1.1	3.9	—	6.96
Calcium caseinate	4.0	91.2	1.2	4.7	—	6.85
Isolated soy proteinate	4.9	92.0	0.4	3.9	0.2	7.04
Soy protein concentrate	7.8	65.1	0.4	4.7	2.9	6.92
Defatted soy flour (Toasted)	6.3	52.8	1.0	6.0	2.4	6.82
Peanut flour	7.1	48.2	9.3	4.1	3.0	6.75
Fish protein concentrate	2.1	78.2	0.4	19.0	—	6.50
Cottonseed flour	9.6	54.5	2.4	6.9	3.2	6.61

¹All values expressed as % on an "as is" basis and represent the averages of three samples each.

²3% suspension in distilled and demineralized water.

several soy products to > 27% in unextracted whole milk powder. Lipids can have detrimental effects on flavor (15, 17, 18) and hence products such as whole milk powder, buttermilk powder, peanut flour and cottonseed flour should be more susceptible to staling and rancidity.

Ash values were similar for all products except the higher value for fish protein concentrate.

Fiber content can be detrimental in processing and in acceptability, especially in drinks formulated from supplements. For example, products with fiber present may clog processing filters and damage equipment. Also, if not properly processed, liquid products containing fiber may form an objectionable sediment upon standing. Thus, milk-based supplements have a definite advantage since they contain no fiber. Although fish protein concentrate contains no fiber, its high ash content can be detected as particulate in some foods.

The pH values ranged over approximately 1 unit, with a low of 6.06 for whey powder and a high of 7.04 for isolated soy proteinate. Besides the more acidic flavor characteristic of lower pH values (1), pH can also be associated with solubility of the protein which in turn affects product functionality and acceptability (7).

Sensory evaluation

A statistical summary of the sensory panel results are presented in Fig. 1, 2, 3, and 4. If odor intensity of powder alone is considered (Fig. 1), no statistical difference ($\alpha = 0.05$) was found among milk products. Within this milk-based group, odor intensity of low heat NFDM powder was judged most bland and that of whole milk powder the least bland. Also, the odor intensity of powdered sodium caseinate was judged slightly better than that of calcium caseinate. Mineral reduction in whey powder also resulted in a blander smelling product. Among the odor intensities of vegetable and marine products, cottonseed flour was statistically blander than peanut flour and fish protein concentrate. Among classes, cottonseed flour was judged the most bland in odor intensity and isolated soy proteinate more bland than either milk caseinate. In summarizing, when using low heat NFDM as the control, all products except defatted soy flour, peanut flour, and fish protein concentrate were statistically the same in odor intensity in the powdered form.

From Fig. 2 it can be seen that the odor intensities of liquids essentially followed the same general pattern as those of powders. Again, there was no statistical difference among products within the milk group. However, the liquid vegetable and marine products generally were judged less bland in odor

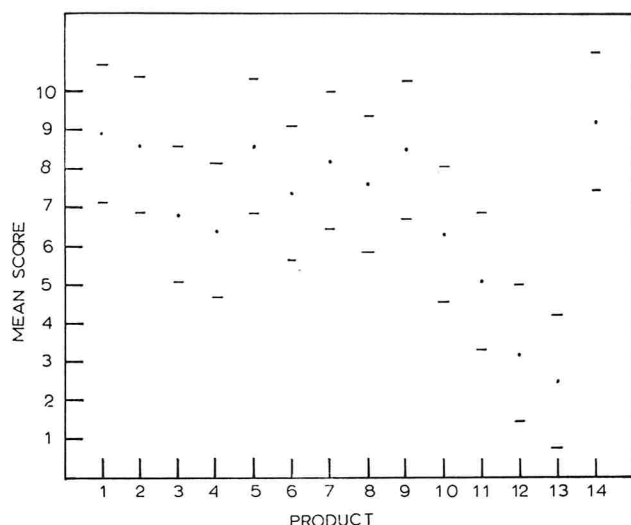


Figure 1. Honest significant difference intervals ($\alpha = 0.05$) for odor intensities of powders. Products: 1. Low heat NFDM, 2. High heat NFDM, 3. Whey powder, 4. Whole milk powder, 5. Demineralized whey powder, 6. Buttermilk powder, 7. Sodium caseinate, 8. Calcium caseinate, 9. Isolated soy proteinate, 10. Soy protein concentrate, 11. Defatted soy flour, 12. Peanut flour, 13. Fish protein concentrate, and 14. Cottonseed flour.

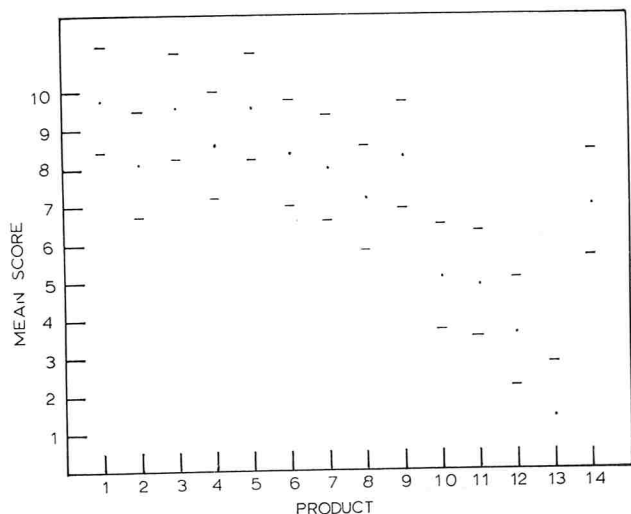


Figure 2. Honest significant difference intervals ($\alpha = 0.05$) for odor intensities of liquids. Products: Same key as in Figure 1.

intensity than were their corresponding powders. For example, the odor intensity of cottonseed flour was judged most bland in the powdered state but was judged tenth most bland in the liquid state. Again, using reconstituted low heat NFDM as the control, all liquid products except soy protein concentrate, defatted soy flour, peanut flour, and fish protein concentrate were statistically the same in odor intensity.

When flavor scores of liquids are considered (Fig. 3), the downward trend of vegetable and marine protein sources continued. The only liquid product in

this class which was statistically equivalent to milk sources, in odor intensity, was cottonseed flour. Within the milk group, again reconstituted low heat NFDM was judged the most bland in flavor intensity. Data on flavor intensity of liquids within the milk group demonstrated that calcium caseinate was statistically inferior in blandness to the other milk products. Thus, with reconstituted low heat NFDM as a control, flavor intensities of liquefied calcium caseinate, isolated soy proteinate, soy protein concentrate, defatted soy flour, peanut flour, and fish protein concentrate were judged statistically inferior.

By combining odor intensity and flavor scores of liquids, an indication of overall sensory acceptability can be obtained. These data are summarized in Fig. 4 and show that all milk-based supplements, with the exception of calcium caseinate, had higher composite sensory blandness scores than any of the vegetable and marine products. Thus, by sensory panel evaluation of both odor and flavor intensities in both powdered and liquid forms, it would appear that milk-based supplements have a definite flavor advantage over the vegetable and marine supplements evaluated.

Headspace investigations

Buttery and Teranishi (6) reported that volatile compounds in foods could be characterized and quantitated just as effectively instrumentally (gas-liquid chromatography, GLC) as organoleptically. Issenberg (12) has also discussed the merits of GLC headspace analysis of volatile food constituents.

It is realized that isolation and GLC operating conditions can affect results. Also, no one GLC column, even if operated over a wide temperature range, can assure complete separation and detection

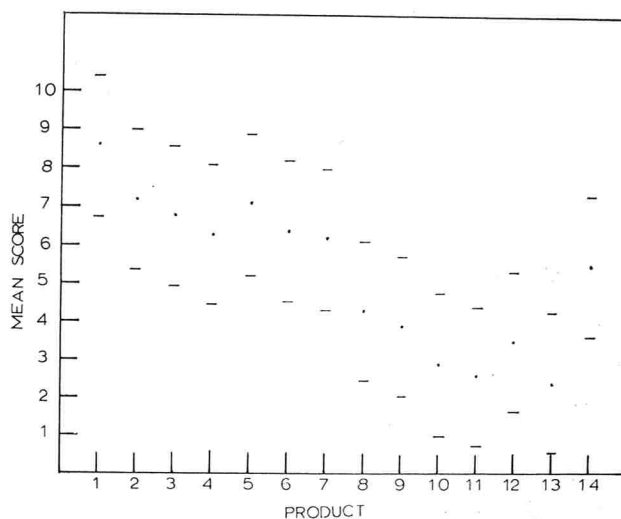


Figure 3. Honest significant difference intervals ($\alpha = 0.05$) for flavor intensities of liquids. Products: Same as key in Figure 1.

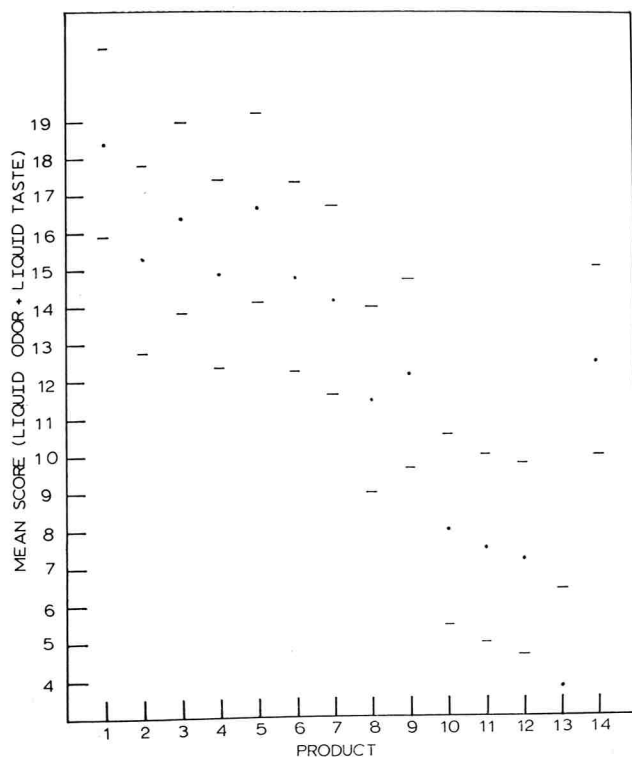


Figure 4. Honest significant difference intervals ($\alpha = 0.05$) for combined odor and flavor intensities of liquids. Products: Same as key in Figure 1.

of all compounds relating to odor and/or flavor. Since this was a comparative study, only a Carbowax 20 M column was employed. It was operated at a relatively low temperature to effectively separate most volatile compounds which are detectable by sniffing at room temperature.

All headspace vapors of the products evaluated produced the same general GLC headspace scan except the more bland products failed to yield all peaks and intensities of remaining peaks decreased. This coincides with the work of Hougen et al. (10) since they found that different cereal grains and oilseeds produced similar vapor profiles.

To quantitate and compare GLC vapor patterns with results of the sensory panel, all peak areas were calculated and totaled for each product. These data are in Fig. 5. Similar general trends can be noted for the instrumental and sensory evaluations. Both techniques demonstrated that the milk-based products, as a class, were more bland than the non-milk supplements. However, within the milk class certain discrepancies appeared. By sensory evaluation, whole milk powder was one of the less bland milk products. However, by GLC technique, whole milk powder appeared to be the most bland milk product. A possible explanation and certainly a major limitation with the direct GLC headspace technique is that a majority of the volatile compounds were fat

soluble and thus not released to be detected by the GLC procedure. Peanut flour was quantitated to contain more volatile compounds than all other samples evaluated. Isolated soy protein was judged by sensory evaluation to be one of the more bland non-milk supplements but GLC headspace analysis revealed it to contain a large quantity of detectable volatile compounds.

Thus, it would appear that limited correlation exists between sensory evaluation and GLC direct vapor response when a variety of products are compared. However, closer correlation between the two techniques would result if processing and storage changes were followed within the same samples.

Carbonyl content

The role of carbonyl composition and content in

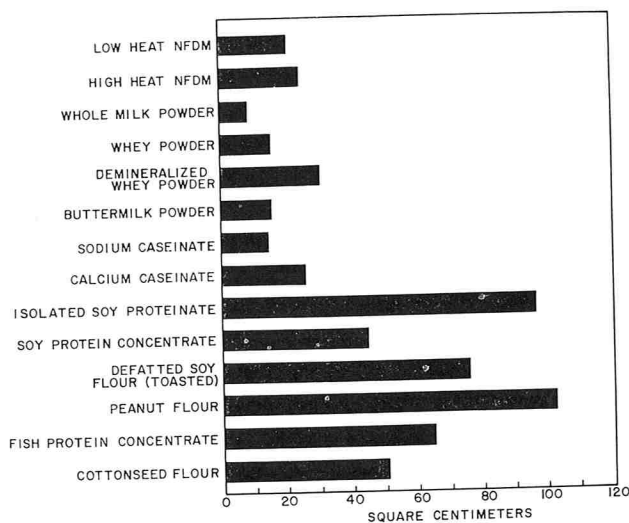


Figure 5. Total GLC headspace peak areas for products evaluated. (Areas for all peaks of each product calculated and summed to total area in square centimeters for each product).

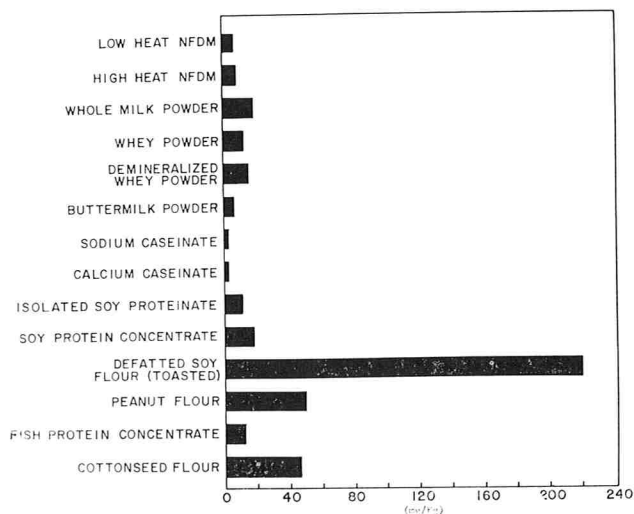


Figure 6. Colorimetric carbonyl content (mg/kg) for products evaluated. (Relative to Butanal Standard).

flavor deterioration of foods is well documented (5, 6, 15). Although all carbonyls do not contribute equally to odor and taste, their level in foods can serve as an indicator of product quality (6). Figure 6 shows the total carbonyl content expressed in mg/kg for the products evaluated. Toasted, defatted soy flour contained the greatest amount of carbonyl compounds. This was probably caused by toasting. Although soy flour is toasted to improve flavor, this improvement results from an increase in carbonyl compounds. Aside from whole milk powder, the milk supplements had carbonyl values indicative of their degree of processing. In general, the non-milk supplements had carbonyl values corresponding to their degree of processing and/or fat content.

In fish protein concentrate, carbonyl content does not appear to be indicative of flavor acceptability since most of the objectionable odors associated with this product are of an amine nature.

Apparently carbonyl content is not a good indicator of total flavor acceptability when working with a wide range of products. As with headspace analysis, carbonyl content is more suitable as an indicator of flavor acceptability resulting from processing and/or changes occurring during storage of a single product.

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A COMPARISON OF TWO AND THREE DAYS INCUBATION FOR ENUMERATING RAW-MILK BACTERIA¹

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ABSTRACT

Eighty-three samples of raw milk were assayed by the Standard Plate Count method with incubation periods of two and three days. The three-day incubation period gave higher counts ($0.05 > p > 0.01$). There were no appreciable problems (such as drying-out, spreaders, molds, etc.) encountered when plates were incubated the extra day. An optional three-day incubation period for the Standard Plate Count is recommended.

Standard Methods, 12th edition, (3) specifies an incubation temperature of 32 ± 1 C for 48 ± 3 hr when assaying milk or milk products for bacteria by the standard agar plate method. The 11th edition of *Standard Methods for the Examination of Dairy Products* (2), also specified a 48-hr incubation period for raw milk but recommended a 72-hr incubation for dried milks. The longer incubation period for the dried milk was advocated by a Committee set up by the International Dairy Federation (see review) (7). The change back to two days was made after studies by Pedraja (9) indicated that al-

though counts were greatly increased after three days of incubation, the grade classification (1) was only seldom influenced. The effect of three days incubation on raw milk bacteria was studied by Babel, et al. (4). These workers found no difference in counts when plates were incubated at 32 C for two or three days but did find higher counts at five days. Pasteurized milk, however, showed counts at three days to be higher than at two days. This was also the finding of Nelson and Baker (8).

It is often necessary or desirable to assay milk samples on a Friday—a two-day incubation time would necessitate making plate counts on a Sunday whereas three days of incubation would permit observations to be made on a regular working day. The study reported here was intended to further investigate the effect of a three-day incubation period on plate counts of raw-milk bacteria with a special emphasis on the possible development of undesirable characteristics in the over-incubated plates which might make enumeration more difficult or less precise. Statistical evaluation of the two incubation periods was made with an analysis of differences of mean counts and variances encountered.

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MATERIALS AND METHODS

The study was conducted by nine subcommittee members, each of whom collected his own raw-milk samples either from farm bulk tanks or from holding tanks at the processing plants. There were two separate analyses, with different milk samples and different analysts from the laboratory of investigator D. These were considered to be of equal weight in the statistical evaluation. Another laboratory, that of investigator G, on the other hand, reported the results of analyses of the same milk samples by two analysts; thus providing an opportunity to determine a possible interaction between analysts and samples. The assay methods were those recommended by *Standard Methods* (3) except that a three-day incubation period was included with the same plates being counted at both two and three days. All plates were poured in duplicate. Statistical evaluation was in general similar to that of previous studies by the Subcommittee (5, 6).

RESULTS

Mean counts

The means of all samples tested are shown in

TABLE I. COMPARISON OF TWO AND THREE DAYS INCUBATION ON PLATE COUNTS

Investigator no.	Milk sample no.	Incubation time		Δ^1 %		
		2 days	3 days			
A	1	4.5	5.2	4.7	5.8	8.2
	2	4.2	6.2	6.5	4.0	1.0
	3	6.5	5.6	6.7	5.8	3.3
	4	9.9	10.0	9.9	10.4	2.0
	5	4.4	6.1	4.8	6.5	7.6
	6	22.7	24.9	23.3	25.8	3.2
	7	6.9	7.7	7.3	7.9	4.1
	8	9.0	9.5	9.3	9.7	2.7
Average		8.96		9.28		3.6
B	9	4.2	3.9	4.5	4.0	4.9
	10	16.0	18.3	17.5	19.5	7.9
	11	11.0	11.8	11.3	12.0	2.2
	12	8.7	8.3	9.4	9.1	8.8
	13	4.8	4.5	4.9	4.8	4.3
	14	15.6	15.1	16.9	16.0	7.2
	15	4.5	4.3	4.7	5.1	11.4
	16	5.1	4.9	5.4	5.3	7.0
Average		8.81		9.40		6.7
C	17	111.0	119.0	106.0	111.0	-5.7
	18	980.0	1000.0	910.0	1100.0	1.5
	19	228.0	286.0	240.0	272.0	-0.4
	20	9.2	12.0	10.2	11.8	3.8
	21	2.9	2.8	3.7	3.6	10.5
	22	4.8	2.8	4.9	3.9	15.8
	23	2.9	4.0	3.2	4.5	11.6
	24	5.8	6.3	6.4	6.8	9.1
Average		173.59		174.88		0.7
D ₁	25	13.7	15.1	13.8	15.4	1.4
	26	8.2	9.9	8.4	9.4	-1.6
	27	239.0	290.0	252.0	284.0	1.32
	28	7.4	7.0	8.5	6.6	4.9
	29	14.7	14.6	14.8	15.1	2.0
	30	8.2	7.5	8.7	7.4	2.5
	31	61.0	67.0	57.0	71.0	0
	32	24.7	24.6	24.0	27.3	4.0
Average		50.79		51.46		1.3
D ₂	33	6.4	7.3	7.0	7.6	6.6
	34	12.1	11.5	12.2	12.8	5.9
	35	7.2	9.2	7.1	9.7	2.4
	36	31.0	49.0	42.0	47.0	11.2
	37	21.0	19.5	20.0	22.6	6.4
	38	4.0	3.8	5.3	5.2	34.6
	39	14.2	13.7	15.0	14.0	3.9
	40	9.2	8.9	9.8	9.7	7.7
Average		14.25		15.44		8.4
E	41	13.4	11.0	10.7	13.3	-1.6
	42	6.0	5.4	7.4	5.8	15.8
	43	7.1	6.8	7.2	6.9	1.4
	44	85.0	96.0	85.0	109.0	7.2
	45	2.3	2.6	2.3	2.7	2.0
	46	127.0	129.0	124.0	132.0	0
	47	9.9	10.1	11.2	11.3	12.5
	48	140.0	130.0	145.0	144.0	7.0
	49	40.0	36.0	47.0	37.0	10.5
	50	13.7	15.2	14.5	15.9	-4.7
	51	615.0	650.0	647.0	650.0	2.5
	52	27.0	29.0	25.5	27.5	-5.4
Average		92.10		95.09		3.2
F	53	22.7	22.5	23.5	23.4	3.8

	54	8.7	10.0	9.3	11.0	8.6
	55	7.9	9.4	9.4	9.8	11.0
	56	3.8	3.4	4.4	3.5	9.7
	57	1.3	1.6	1.4	1.8	10.3
	58	5.6	5.6	6.5	5.8	9.8
	59	4.1	4.1	4.3	4.2	3.6
	60	3.3	3.4	4.1	3.7	16.4
Average			7.33		7.88	7.5
G ₁ ²	61	203.0	210.0	236.0	235.0	14.0
	62	1.3	0.8	1.6	1.3	38.1
	63	9.1	9.6	9.7	10.3	7.0
	64	127.0	146.0	163.0	156.0	16.8
	65	8.1	7.8	9.1	10.2	21.4
	66	44.0	72.0	51.0	71.0	5.2
	67	10.0	10.5	11.7	14.5	27.8
Average			61.37		70.03	14.1
G ₂	61	221.0	204.0	246.0	219.0	9.4
	62	2.6	2.0	2.5	2.0	-2.2
	63	10.8	12.0	12.6	12.5	10.1
	64	143.0	217.0	171.0	232.0	11.9
	65	9.1	10.1	10.4	11.0	11.4
	66	66.0	56.0	72.0	63.0	10.6
	67	14.7	12.1	15.1	14.4	10.1
Average			70.31		77.39	10.5
H	68	5.9	3.3	6.2	4.8	19.6
	69	7.3	6.2	6.9	6.2	-3.0
	70	6.7	6.9	7.7	8.0	18.0
	71	10.9	12.3	11.5	12.6	3.9
	72	4.0	4.0	5.6	5.6	40.0
	73	39.0	38.0	43.0	42.0	10.3
	74	10.1	10.4	13.7	13.8	34.1
	75	13.3	13.0	14.5	13.4	6.1
Average			11.96		13.47	12.6
I	76	65.0	35.0	66.0	35.0	1.0
	77	7.6	8.6	7.1	10.3	7.4
	78	125.0	123.0	117.0	127.0	-1.6
	79	130.0	136.0	170.0	132.0	13.5
	80	87.0	86.0	97.0	120.0	25.4
	81	21.0	22.2	22.2	22.0	1.8
	82	23.6	26.2	26.7	23.3	0.4
	83	23.5	22.5	23.3	24.0	2.8
Average			58.89		63.92	8.6
Overall Average			52.24		54.88	5.0

¹Change (Δ) was positive unless otherwise noted.

²Analysts G₁ and G₂ took different aliquots of same milk samples and plated and counted independently. For actual counts multiply by 1000.

Table 1. There was an increase in plate counts at three days with each investigator with the increases ranging from 0.7% for investigator C to 14.1% for investigator G₁. The overall mean difference was 5.0% in favor of the three-day incubation period. Only nine of the analyses showed lower counts at three days than at two. This included the results of analysts G₁ and G₂ who assayed the same milk samples. The greatest individual sample gain was 40% with investigator H and milk sample number 72. Analysts G₁ and G₂, although assaying the same milk samples (using separate dilutions and plates), showed differing degrees of change from two to three days

incubation. In one instance, milk sample number 62, a decrease of 2.2% was noted for one analyst and an increase of 38.1% for the other.

Statistical evaluation of plate counts

A non-parametric sign test was made counting the number of times three days incubation was superior to two days and the number of times two days was superior. The test showed that the increase in three days was significant at the 1% level of probability.

An analysis of variance of the log₁₀ transformed counts is shown in Table 2. The largest source of variation was in the milk samples themselves; the differences were significant with $p < 0.01$. The

TABLE 2. ANALYSIS OF VARIANCE OF PLATE COUNTS

Source of variation	df	Sum of squares	Mean squares	F ratio ¹	Significant at	
					p < 0.05	p < 0.01
a Investigators	9	24.7931	2.7547	2.27	yes	no
b Samples within investigator	73	88.4660	1.2118	504	yes	yes
c Days	1	0.0869	0.0869	6.6	yes	no
d Days times investigators	9	0.1191	0.0132	13.2	yes	yes
e Days times samples within investigators	74	0.0779	0.0010	0.42	no	no
f Error (between duplicate plates)	166	0.4114	0.0024			
Total	331	113.9544				

¹F ratios obtained using lines a/b, b/f, c/d, d/e, e/f

TABLE 4. ANALYSIS OF VARIANCE SUMMARY OF VARIANCES DUE TO INVESTIGATORS AND DAYS OF INCUBATION

Source of variation	df	Sum of squares	Mean squares	F ratio	Significant at	
					p < 0.05	p < 0.01
Investigators	9	1.9808	0.2201	4.32	yes	no
Investigator B vs. others	1	1.0775	1.0775	21.13	yes	yes
Days	1	0.0108	0.0108	<1.0	no	no
Days times investigators	9	0.5101	0.0510			
Total	19					

treatments (days) showed a significantly higher count ($p < 0.05$) at three days than at two. Investigators were also significantly different with $p < 0.05$ but not with $p < 0.01$. There was a significant interaction effect ($p < 0.01$) between days of incubation and investigators.

Statistical evaluation of variabilities

Table 3 shows the averages of single-degree-of-freedom variances for days of incubation and investigators. Investigator B in previous experiments showed the lowest variability of any other investigator; this was also true in these studies. A further study of the significance of these variance differences was by an analysis of variance of \log_{10} transformed variances of Table 3. The results are summarized in Table 4. The variances (an indication of reproducibility) were not different for two or three days incubation but the investigators did show significant differences in reproducibility with $p < 0.05$ but not with $p < 0.01$. Since most of this difference was suspected as being the low variance (high reproducibility) of investigator B, an orthogonal contrast was made between this investigator and the other nine. The F ratio of this contrast was significant with $p < 0.01$ indicating that this investigator has a lower variability than the others.

Each investigator was given a form to fill out with space for comments on any difficulties encountered (such as drying-out, spreaders, molds, etc.) by incubating the plates for an extra day. No com-

ments were made by six of the participants indicating, presumably, that they encountered no problems. One investigator reported a slight increase in spreaders in two of the plates and slight dehydration in a third but these did not interfere with the counting procedure. Two other investigators reported some mold growth at three days in one sample but again, counts were reported without any apparent difficulties encountered. Thus, out of the 83 milk samples and 166 plates only five plates showed signs of conditions which might interfere with normal counting procedures. One investigator reported that counts

TABLE 3. VARIANCE ESTIMATES OF PLATE COUNTS¹

Laboratory number	Incubation period		Average variance
	Two days	Three days	
A	0.003825	0.004862	0.004344
B	0.000462	0.000475	0.000469
C	0.006238	0.002912	0.004575
D ₁	0.001200	0.002288	0.001744
D ₂	0.003538	0.001638	0.002588
E	0.000908	0.002133	0.001521
F	0.001250	0.001988	0.001619
G ₁	0.006800	0.002914	0.004857
H	0.004500	0.001100	0.002800
I	0.004912	0.007962	0.006437
Average variance	0.003363	0.002827	0.003095

¹These variance estimates (standard deviation squared) were calculated from the pooled single degree-of-freedom variances between duplicate plates using \log_{10} counts.

were easier the third day, since the colonies had increased in size. Another reported that pinpoint colonies had developed on the third day which were not apparent on the second day of incubation. It appeared that incubation for the extra day did not cause any appreciable difficulty in the standard plate method.

DISCUSSION

The results of this experiment showed that there were great differences between investigators and between milk samples in the variations observed. These same variations were observed before (5, 6) and em-

phasize the need for enlisting the help of several laboratories, each assaying a number of milk samples, when changes in analytical methods are being contemplated. The increase in average counts at three days between the different laboratories participating in this study ranged from a negligible 0.7% for investigator C to a considerable 14.1% for investigator G₁. Erroneous conclusions although not serious in this instance, could easily have been reached if investigator C, for instance, had been the only participant. There was a significant interaction between treatments (days) and investigators in this study but no interactions between days and samples within investigators. Previous reports (5, 6) indicated significant interactions between investigators and treatments and between treatments and samples within investigators.

The results of analysts G₁ and G₂ also indicated that different investigators may get different treatment effects from the same milk samples, however, it should be noted that the most striking example of analyst differences was with milk sample number 62 which had the lowest count of the seven tested. The plate counts of this sample were < 30 colonies per plate and large inaccuracies would be expected. If enough random samples are chosen these effects can be weeded out and successful conclusions can be made.

We would recommend on the basis of these studies

that an optional three-day incubation period be allowed for the standard agar plate method for enumerating raw-milk bacteria. The 5% difference in counts at three days would ordinarily be well within the limits of experimental error.

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Errata

**PREDICTION OF STANDARD PLATE COUNT OF
MANUFACTURING-GRADE RAW MILK FROM THE PLATE
LOOP COUNT**

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This paper appeared on pages 168-170 of volume 33 (April, 1970) of the *Journal of Milk and Food Technology*. Errors occur in Tables 3 and 4. The errors in Table 3 are in column 2 (predicted standard plate count $\times 10^4$). Errors in Table 4 were also in column 2 (predicted SPC $\times 10^4$). Corrected tables appear below. Values in Table 3 are taken from a reliable trial using a different set of data than was used for the initial analysis.

TABLE 3. A COMPARISON OF THE PREDICTED AND ACTUAL STANDARD PLATE COUNTS

Plate loop counts $\times 10^4$	Predicted standard plate count $\times 10^4$	Standard plate count $\times 10^4$
52	61	67
53	62	40
54	63	54
54	63	60
57	67	70
58	68	51
61	72	120
62	73	100
62	73	90
62	73	70
62	73	68
64	76	100
65	77	97
65	77	72
65	77	80
67	79	78
68	81	110
70	83	110
70	83	80
73	87	100
73	87	81
74	88	100
78	93	100
78	93	92
78	93	100
78	93	92
83	99	100
84	100	150
87	100	120
89	110	100
91	110	130
93	110	120
97	120	140

100	120	120
110	130	110
110	130	100
120	150	190
120	150	120
120	150	220
120	150	120
120	150	110
130	160	200
130	160	150
140	170	200
140	170	180
150	180	320
150	180	200
170	210	210
170	210	190
190	230	310
230	290	410
240	300	350

TABLE 4. CHART FOR CONVERTING PLC TO PREDICTED SPC

PLC $\times 10^4$	Predicted SPC $\times 10^{4*}$
23 - 30	30
31 - 38	40
39 - 47	50
48 - 55	60
56 - 63	70
64 - 71	80
72 - 79	90
80 - 87	100
88 - 95	110
96 - 100	120
110	130
120	150
130	160
140	170
150	180
160	200
170	210
180	220
190	230
200	250
210	260
220	270
230	290
240	300
250	310

*Rounded to nearest 10,000

DAIRY FOODS: TRENDS, RESEARCH, AND DEVELOPMENT

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ABSTRACT

The status and trends within the dairy industry are reviewed in relation to the current concern with new product development. The expediency of devoting further research toward improving the technology and quality attributes of traditional products for which there is a growing demand, i.e., low-fat beverage milks, cheeses, etc., is emphasized. The need to apply practical research findings in the formulation of dairy ingredients for other sectors of the food industry are cited especially with regard to the manufacture of milk powders for the bakery industry.

Today the food industry is both actively and articulately involved with new product development (2, 5, 8). This includes new formulations, imitation foods, and new items and they are designed to compete with or replace existing products based on their superiority in convenience, cost, and quality (4, 5, 6, 9). The fabrication, packaging, and promotion of new foods requires a high level of technical skill and a thorough knowledge of the physical nature and chemical interactions of ingredients under various conditions of processing and storage. Consequently the modern food industry is creating a growing demand for finer ingredients of known composition and functionality. Coupled to this new demand is the development of new, cheaper sources of good quality food proteins from oilseeds, cereals, and eventually perhaps from leaves and microbial sources (8, 9). Progress in food development and the concomitant investigation of new proteins has increased the competition between modern food commodities and some long established products, e.g. dairy products and meats. Because of this progress in the food industry, there is much concern for the fate of several segments of the dairy industry. Much has been written concerning the vital role of new product development in maintaining the market popularity of dairy foods (16). The present article examines the status of this industry and discusses the present preoccupation with product development.

The dairy industry is very healthy and its basic product commodities are maintaining relatively stable consumption levels. However, the rate of expansion has been limited when compared with the multiplicity and variety of products developed by other commodity groups within the food industry. The conservative nature of the traditional dairy industry and

restrictive legislation may be specified as the main reasons for retarding innovation. However active involvement in uncertain and expensive product development should be evaluated critically because it has been estimated that it can cost up to 10 million dollars per product to develop an idea into a super-market commodity. The structure and production trends of the industry warrant examination because this may clarify a rationale for establishing present and future priorities and indicate the important and specific areas for new product development in the modern dairy industry.

STATISTICAL TRENDS

Milk production, which peaked at 127 billion lb. in 1964 has been slowly declining and in 1969 output was 116 billion lb. This has corresponded to a decrease in the number of dairy farms and cow population which in 1969 was 12.7 million. The quantity of milk produced per cow attained a record high of 9,150 lb. Dairy farm income, based on milk production, was approximately \$6.2 billion in 1969. The milk was disposed of in the following categories: beverage milk and cream, 47.5; butter, 20.7; cheese, 16.5; ice cream, 9.5; evaporated and condensed milk, 3.4; milk powders, sour cream, yogurt, etc., 2.6%, respectively (7). Based on 'average' prices, the approximate retail value of dairy goods was around \$14 billion in 1969. Preliminary 1970 statistics indicate that 50% of the 118 billion lb. of milk produced was used in manufactured dairy products. Its disposal in various products was comparable to 1969 except that most cheeses, particularly Cheddar, Swiss, and Italian types were produced in increasing quantities (18).

These disposition data are very useful when methods and means for improving quality and increasing sales of specific dairy products are being considered. The statistics indicate the particular products which are the major components of the industry and they indicate the logical commodity areas in which to promote and expand sales. However, before discussing such activities it may be expedient to review recent trends in consumption of individual commodities (3, 7, 18).

Sales of fluid whole milk are declining very slightly

TABLE 1. IMPORTS OF SOME DAIRY PRODUCTS
IN YEARS 1967-1969

	Thousand pounds			Value \$ × 1000
	1967	1968	1969	
Dried skimmilk	924	1,747	1,914	(209)
Dried buttermilk	158	456	174	(24)
Butter	677	739	678	(367)
Butterfat mixtures	100,548	1,882	2,741	(740)
Swiss cheese	14,355	38,851	20,108	(10,600)
Gruyere cheese	9,836	19,977	12,650	(6,329)
Cheddar	4,967	9,842	9,606	(3,840)
Blue mold cheese	4,788	4,823	4,877	(2,773)
Gouda & Edam cheeses	11,615	21,386	11,457	(5,306)
Colby	55,230	5,859	6,033	2,046
All cheese total	151,780	170,250	144,102	(68,224)
Casein	99,670	115,092	116,107	(25,428)
Whole milk equivalent of total imports (billion lb.)	2.9	1.8	1.6	

from a per capita consumption of 246 to 220 pints between 1965 to 1969. On the other hand, sales of low fat and fluid nonfat milk are rising rapidly from 31.5 to about 50 pints in the same period. Thus overall, the consumption of beverage milk has stabilized in recent years.

Consumer demand for cheeses continues to increase at a marked rate and in the decade preceding 1970 domestic consumption expanded by about 40% and reached 10.6 lb. per capita in 1969. In addition, sales of cottage cheese are increasing and attained a per capita level of 5 lb. in 1969. Yogurt sales are also expanding rapidly and reached 143 million lb. in 1969. Consumption of ice cream and frozen dairy desserts has remained quite constant since 1965 at approximately 64 lb. per capita expressed as milk equivalents.

Sales of high fat dairy products, butter and creams, continue to decline and presently per capita consumption of butter is 5 lb. per annum, down approximately 30% since 1960. In the same period sales of fluid cream dropped from 9.3 to 5.9 lb. in 1969. On

the other hand, the sour cream market continued to expand.

Civilian consumption of nonfat dry milk has maintained a level of around 1.1 billion lb. in the last decade and per capita consumption was 5.7 lb. in 1969 (3). However, production of this powder has been decreasing in recent years and 1.45 billion lb. produced in 1969 showed a decrease of 9.6% from 1968. Surpluses of this commodity are almost exhausted. Many end-use markets for nonfat dry milk decreased in 1969 except that packaged for home use, dry mixes, and soft drinks. Dry whole milk production continued to decrease from 94 to 75 million lb. between 1968/1969 though total domestic and per capita consumption has shown a fluctuating pattern in the past decade. Dry buttermilk production decreased from 76 million lb. in 1966 to 66.5 million lb. in 1969, whereas whey powder production is rapidly expanding and attained a level of 500 million lb. in 1969. Production of whey powder for human use expanded markedly in 1970 (18). This continuing increase can be attributed to improved manufacturing technology and to scientific research which demonstrated the nutritional value and functional properties of whey and its potential application in various formulated foods.

These statistics reveal that the domestic consumption of many dairy products is expanding and, in reality, sales of only butterfat and creams have shown marked diminution in the last decade.

Since production of milk has been decreasing slightly while consumption is expanding, the large amounts of surplus dairy products have diminished and commodity credit corporation butter stocks decreased from 150 to 64 million lb. between 1967 and 1969. These trends were helped by increased exports in this period. Stocks of dry milk powder have decreased also. The combination of decreasing production and low stocks is significant if concerted

TABLE 2. AMOUNT OF MILK POWDERS USED BY SOME MANUFACTURING SEGMENTS OF THE FOOD INDUSTRY

	1967		1968		1969	
	Pounds (million)	Per cent of total	Pounds (million)	Per cent of total	Pounds (million)	Per cent of total
<i>Nonfat dry milk</i>						
Bakery	250.0	26.5	236.7	23.0	187.0	18.4
Prepared mixes	69.0	7.3	79.6	7.7	101.7	10.0
Confectionery	18.0	1.9	24.8	4.9	19.3	4.4
<i>Dry whole milk</i>						
Bakery	4.6		5.6		4.0	
Candy	36.2		44.1		37.3	
<i>Dry buttermilk</i>						
Bakery	24.2		22.2		21.4	
Prepared mixes	19.3		19.2		22.9	
Candy	4.1		6.3		3.0	

TABLE 3. ACTUAL AND ANTICIPATED GROWTH OF BAKERY PRODUCTS 1958/1980⁵

Category:	A ¹				B ²				C ³				D ⁴			
	1958	1967	1970	1980	1958	1967	1970	1980	1958	1967	1970	1980	1958	1967	1970	1980
Population (m)	175	199	206	235	175	199	206	235	175	199	206	235	175	199	206	235
Consumption (lb. per capita)	68.6	72.6	72.8	76.8	10.2	10.1	10.1	10.1	16.3	17.0	17.4	18.1	13.4	18.0	20.0	24.6
Production (billion lb.)	12.0	14.5	15.0	18.0	1.78	2.01	2.08	2.37	2.84	3.38	3.60	4.25	2.35	3.57	4.12	5.78

¹A - Bread type products: includes white pan bread, rolls, variety breads, hamburger and weiner and other rolls, English Muffins, crumbs and bread stuffing.

²B - Cookies: includes sandwich cookies, marshmallows, wafers, etc.

³C - Sweet goods and desserts: yeast raised donuts, sweet rolls, coffee cakes, soft, pound layer and fruit cakes, pies, pastries and cake donuts.

⁴D - Crackers and baked snacks: Graham crackers, saltines, cracker sandwiches, melba toast, pretzels and other extruded and/or baked snack goods.

⁵Source. Bakery Production, 1971.

programs to enhance sales of dairy products in the U.S. are being planned.

In surveying the dairy industry, the U. S. market must also take cognizance of the world situation (12, 20). World production of dairy produce continues to increase, especially in Europe where large surpluses have accumulated and are causing serious disposal problems. Butter production increased by 7% in 1968 and preliminary 1970 stocks were calculated at 900 million lb. Consequently, the European economic community is subsidizing exports of butter and milk powders. In fact, butter is available for as low as 13 cents per pound for food manufacturers processing foods for export.

World trade in dairy products increased 35% in the last decade and was equivalent to 54 billion lb. of milk (20). The geographic market, however, is mainly limited to the affluent nations, and Europe is now a net exporting area. The prospective markets are in Asia and perhaps Africa though real demand is limited in these areas. Presently the Oceanic countries are actively promoting the Oriental market.

The U. S. imports an appreciable volume of dairy products (Table 1) with cheese and casein being the major items (12). Exports decreased in 1969 to 0.9 billion lb. (milk equivalents) compared to 1.2 billion lb. in 1968 mainly because of the decline in butter shipments under PL480. Exports of dried milks and cheese increased slightly. There was a net importation of approximately 0.7 billion lb. of milk equivalents in 1969 or approximately 0.5% of domestic production, a negligible quantity in terms of competition on domestic market and international economic goodwill.

With this background information, examination of potential areas of expansion can be effected more realistically. Obviously limiting imports of dairy

products will not markedly expand domestic sales, though its impact on certain segments of the industry, e.g. some cheeses, might be considered significant. Secondly, production of raw material, i.e. milk, has reached a plateau and presently (and perhaps to an increasing extent in the future) most of this is utilized by the domestic market. Thus in planning an expansion program for dairy products one must be cognizant of the present trends in production and the time required to increase productivity by increasing the population of milk cows. Thirdly, the priorities or order of emphasis should be clear when deciding on programs designed to increase sales of particular commodities. There is little point in developing new commodities that are unacceptable because of cost. Through expediency the dairy industry should primarily be concerned with maintaining and improving the quality of its current successful dominant products, i.e., fluid milk, cheeses, milk powders, ice cream, cottage cheese, and yogurt. These commodity sections of the industry must be strongly established to facilitate extensive and expensive product development. Accomplishing a small increase in the per capita consumption of fluid milk is a far superior investment than producing a "new," but successful product of limited sales. The trends and changes in commodity utilization are worth noting in planning areas of research priorities (2, 3, 4, 6, 8, 9, 16). Thus, the expanding demand for cottage cheese and yogurt would suggest greater emphasis on these commodities compared to fluid cream or condensed milk, the sales of which are decreasing.

RESEARCH

The annual sales value of dairy products has been estimated around \$13,000 million and only 0.1% of this income was invested in research and develop-

ment (R&D) activities in 1968 compared to the 3% average industrial R & D expenditure (2). Furthermore, this R & D money was spent mostly on product modification rather than on fundamental new dairy products. The dairy industry *in toto*, because it has long enjoyed a dominant and deserved position in the food industry has acquired a reputation of conservatism in supporting research and product development. In many instances, instead of exploiting the advances in food science and technology and technical expertise to enhance its products, it has had recourse to restrictive and protective legislation (9, 13). This attitude was aided by the structure of the industry—a multitude of small plants mostly interested in processing a limited number of products with little knowledge or interest in the development of new products or diversification.

However, a new attitude is beginning to pervade the dairy industry. This can be attributed to a number of interrelated factors. The structure of the industry has changed. The many dairy plants have been replaced by larger integrated dairy conglomerates. These latter large companies are staffed with highly competent technical personnel who are very conversant with the principles of food science, technology, and product development. They are aware of economics, the role of individual ingredients in product formulations, and do not have traditional ties to any raw material source. Furthermore, personnel of the dairy industry are now consciously aware of progress that has been achieved by other facets of the food industry through innovative application of research and development and are realizing that restrictive legislation is now hampering rather than helping the dairy industry to compete with new products. Also, it has been recognized that, in addition to maintaining its position as an excellent product, milk for food product formulations must encounter increasing competition from other raw material sources, e.g. soy protein. During the last decade the dairy industry has become closer to the food industry and its identity as a separate entity within the food industry is no longer marked. Consequently, the principles governing actions and developments of the food industry as a whole will apply increasingly to the dairy industry. Thus, the concept of milk as a processable raw material is accepted and its continued use will depend on its functional qualities, price, and consumer appeal.

The dairy industry has recognized the need for a new orientation, and plans to support more research and development during the next few years. Financial requirements for productive research and development reportedly should total \$18 million by 1975 (2). Several large food companies have their own

strong progressive research programs concerning development of new foods and dairy items and the evolution of whey as a major functional food ingredient is largely attributable to industrial efforts.

Any program intended to advance the sale of dairy products in the U. S. might arbitrarily be divided into two major categories, i.e. (a) that concerned with improvement of quality and convenience of established dairy products, and (b) that concerned with the development of new products based on fluid milk, e.g., instant breakfast, and superior functional ingredients such as specific whey powders for use in food manufacturing. Both of these categories would have much in common in the realm of research involving basic chemical and physical properties of components and their interactions during processing and storage.

Resources being spent by the dairy industry should be allocated as expeditiously as possible and no one interest group should monopolize the available funds. New product development should not be misconstrued to rationalize new formulations.

Fluid milk

With regard to established products, continuing emphasis must be placed on fluid milk quality. Beverage milks comprise the major component of dairy sales and consequently warrant active attention in evolving new processes to enhance quality and expand consumption. Generally, the quality of market milk today is good. However, off-flavor problems still occur and must be consistently prevented in the future to maintain consumption levels. Measures to accomplish this include improved quality control at source and may entail processing changes and legal alterations to permit addition of antioxidants, surface active membrane stabilizers, and flavoring substances. Low fat milks are gaining in sales volume because of consumer preferences. This trend should be fully exploited by maintaining flavor quality and emphasizing nutrition by appropriate promotional efforts. Current popular concern with nutrition and malnutrition provides an excellent background for educated promotion emphasizing the balanced nature of milk as a convenient source of nutrition.

Cheese

The demand for various cheeses is increasing and their manufacture is technically easy and most methods can be automated. Cheese production provides an acceptable mode for utilization of milk fat and its only by-product, whey, is becoming very popular in several areas of food manufacturing. The potential for expansion of the cheese market is excellent and it entails a minimum of change in the structure and routine of the dairy industry. However the quality of many cheeses could be improved and many commercial cheeses have not matured sufficiently when

marketed. The growing pressure for rapid production and automation of cheese manufacture has focussed on the need to accelerate the chemistry and biochemistry of the cheese ripening process. Consequently more research is mandatory if the processor is to produce cheese of quality (flavor, color, texture) by accelerated and continuous processes.

Milkfat

The consumer demand for butter is expected to decrease below its present level and there is little likelihood of butter consumption increasing. If low fat milk products continue to gain in popularity an excess of milkfat will be produced and this fact must always be recognized in developing new products. Expansion of the demand for whole milk, cheese, ice cream, creamed cottage cheese, etc. obviates the problems of surplus milkfat. However, milkfat will continue to be a major product and its profitable utilization will challenge the ingenuity and resourcefulness of the dairy scientist for some time (15, 16). The geneticist and nutritionist will perhaps be obliged to improve protein production and endeavor to lessen fat production by appropriate breeding and feeding. It behooves the manufacturer to investigate and develop new processes for milkfat utilization. Appropriate alterations in existing legal standards, pricing, and regulations may also be necessary.

Uses as food ingredients

The food ingredients sector of the food industry offers the dairy industry potential markets for expansion in the area of high protein dairy powders, especially nonfat dry milk and whey powder. Dairy powders are used in many food manufacturing processes—breadmaking, soups, confectionery, candy, meat processing, etc. Presently dairy powders are facing increasing competition from other protein sources, mainly soy proteins. Milk proteins have enjoyed the advantages of better flavor, excellent nutritional quality, and functional properties superior to those of the other proteins. However, oilseed proteins of acceptable flavor, with excellent functional properties, and somewhat lower in costs present an evergrowing competition. It behooves the dairy industry to retain its fraction of the ingredients market by maintaining a high quality product that is regularly available. It should closely coordinate its method of production with the particular requirements, e.g. high heat powder for the bakery industry. Furthermore promotion of its product on the basis of functional properties, consumer preferences, nutritional value, and price per unit of protein is important.

The expediency of maintaining the milk powder market becomes more obvious if one examines the

current trends in the baking industry (1). This industry represents a significant outlet for milk powders (Table 2) (3). In the decade 1958-68 bakery product consumption showed an overall 32% growth rate with per capita consumption increasing in several categories (Table 3) particularly quality rolls, crackers, and baked snacks (1). It has been estimated that the demand for bread type products will increase by 3.5; cookies by 0.4; sweetgoods and desserts by 1.0; and crackers and baked goods by 2.2 billion lb. during the seventies. This means that a total of approximately 30 billion lb. of bakery products will be produced annually by 1980. These trends, gradual automation within the bakery industry, and a continuing obligation to minimize cost of production are of significance to the dairy industry. Generally a maximum of 6% (based on flour weight) nonfat milk powder is added to bread and bakery formulations. This is necessary because it performs several important functions during dough formation, baking, and storage and it enhances the flavor and nutritional value of bread (14). Thus the amount of milk powder used by the bakery industry is of major importance to the dairy industry. It represents approximately 25% of the nonfat dry milk and the preponderance of the buttermilk market. The dairy processor must be continually aware of the trends in other facets of the food manufacturing industry and be willing to amend processes and formulate ingredients and new products as required by buyers.

As the functional prerequisites of the various ingredients used in baking become better understood the search for cheaper but equally functional components will intensify. Refined soy protein preparations are providing increasing competition in the sector of the bakery industry supplied by the dairy industry. The dairy suppliers must ensure that the bakery requirements are fulfilled with the best quality product.

Another factor which will greatly affect the milk powder market is growing automation within the baking industry. The continuous breadmaking process is now used in the manufacture of over 40% of white bread. With this process the normal maximum of 6% milk powder cannot be added to the dough formula because it results in bread of inferior volume and texture. Reasons for the deleterious effects of the milk powders in this process are not known though several have been postulated (14). This problem certainly warrants immediate investigation by the dairy industry because if unsolved, it may cause a significant reduction in the amount of milk powder used in manufacture of bread.

The findings that surface active glycolipids greatly increase the tolerance of doughs to increased quan-

tities of added proteins, including nonfat dry milk powder, is very significant (17). The dairy powder producer should explore the market potential of providing powders with the appropriate surface active agents already added.

The market demand for prepared dry mixes is expanding rapidly and this also provides a good outlet for dairy powder, but it will meet increasing competition from soy proteins. The possibility of producing blends of appropriate combinations of milk powder, eggs, emulsifiers, and shortening for dry mixes should be examined. Spray dried butters and cream (10, 19) should be useful to the soup, baking, and confectionery industries and provide another outlet for butterfat.

Ice cream

In discussing conventional products, the ice cream market also is most promising. This is a commodity of universal appeal, whose edible quality tends to improve with its butterfat content. In 1969 Americans consumed an average of 64 lb. of milk equivalents as ice cream and ice milk (7). The upward trend in consumption may be continued by product and packaging innovations, improved distribution, and effective promotion.

IN CONCLUSION

The preceding discussion briefly suggests some of the principal areas where the dairy industry can expand sales of its established products with a minimum of innovation. Greater emphasis on the various chemical, physical, processing, and packaging factors which affect the consumer's idea of quality and more informative advertising should expand sales in the future.

Finally, new product development is essential though it is expensive and of uncertain success. Several aspects of new product development, pertaining to dairy products, have been thoroughly discussed

in a number of recent articles (2, 4, 5, 6, 8, 9, 11, 13, 15, 16).

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MICROBIOLOGY AND QUALITY ASSURANCE OF PRECOOKED FROZEN FOODS¹

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ABSTRACT

Production of high quality precooked, frozen foods requires constant vigilance by the producer, handler, and user. The history of quality assurance is described. A program of total quality assurance for the food processor is detailed. Environmental sanitation and microbiological control is discussed.

For the last 10 years a change has occurred in both life style as well as food consumption patterns of the American public. This has been brought about by mobility, better income and education, the increased role of women as a working force, and the continuing trend on the part of the consumer to eat out. At the present time the size of the convenience food market is put at 36 billion dollars. Market analysis has shown that foods with built-in convenience and quality grow at a much faster rate than other commodity classes (7). The wide distribution of convenience foods throughout our country which, if unsafe could affect large segments of the population, has brought about intense regulatory interest in this area. A product can be considered in violation of the Federal Food, Drug, and Cosmetic Act and subject to seizure if contaminated with low levels of pathogens such as *Salmonella*. As a result recalls and destruction of contaminated products occur causing an economic loss and loss in sales because of unfavorable publicity. This paper discusses the quality assurance and the microbiological aspects of a segment of the convenience market—precooked frozen foods.

QUALITY ASSURANCE

Production of uniform high quality foods requires both the implementation as well as application of a program of total quality assurance. Historically, this program evolved in five steps. It started in 1900 with "foreman quality control" where the foreman was responsible for the quality of his products. World War I brought "inspection quality control" wherein full time inspectors looked at the product. Sophisticated manufacturing techniques started during

World War II introducing statistics into quality control or "statistical quality control." At present all these concepts are incorporated into a program of "total quality assurance" (5). This program includes all parameters that either directly or indirectly affect product quality. It includes development of "information loops" where problem areas are continuously fed to the responsible departments and promptly solved, thus minimizing substandard product. Any progressive quality control program should put just as great emphasis on prevention of substandard product as rejection of finished packaged product. Further it needs to be dynamic and adaptable to change caused by newer techniques and processes.

An adequate quality assurance program requires not only a continuing investment of money for skilled people and laboratory facilities, but also commitment by management to see it succeed.

PRODUCT DEVELOPMENT AND FORMULATION

Production of consistently high quality products and development of high quality new products stimulates sales and growth of any manufacturing concern. Intelligent formulation and an adequate process needs to be established if a consistent high quality is desired. In an age of automation and mass production one cannot rely on finished testing alone. In the development of potentially hazardous products such as cream puffs or eclaires, safety must be built into the formulation and the process itself. The following factors may be considered important in formulating a product:

pH

It is generally agreed that common types of food poisoning bacteria will not grow in acid foods at pH levels below 4.5; above this pH value growth may occur and is more rapid as the pH is raised (11). Synthetic creams can support bacterial growth and are just as dangerous as "natural" fillings. Crisley et al. (2) and McKinney and Clarke (8) have shown that at room temperature synthetic creams can support bacterial growth. When the fillings contain only water, staphylococci remained viable but did not grow. If water was replaced by milk or if small

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amounts of egg were added, growth took place.

Water activity

The water activity of foods can be a significant factor in retarding growth of microbes. The importance of water activity was shown by Silliker and McHugh (12) who determined that staphylococci did not grow in cream fillings at a sucrose to water ratio of 1.8 to 1. Yet, when these fillings were introduced between layers of devil's food cake they grew at ratios of 2-7 parts of sucrose to 1 part of water. This growth was attributed to the water migration at the interface, the driving factor being the dissimilar water activities of the cake and filling.

Microbial competition

The inherent flora of the food itself may suppress or retard growth of food poisoning organisms. Growth suppression of staphylococci in defrosted chicken pies was observed by Peterson et al. (10).

Quality parameters

Production of foodstuffs that are safe and uniform in quality will require constant monitoring of all parameters that affect finished product quality—namely ingredients, the process of manufacture, finished product, and environmental sanitation.

Ingredients. All ingredients should be purchased on specification and on a continuing Pure Food Guarantee. These specifications should take into account the capabilities of the supplier and the needs of the user. The quality assurance programs should be studied to assure that the supplier is able to deliver uniform flow of quality ingredient. Chemical and bacteriological specifications should define only those parameters that truly reflect quality. As examples, percent fat in milk and percent solids in eggs are good indicators of adulteration. Recent advances in chromatography have enabled determination of the bean strength used in vanilla extracts (13). All ingredients should be systematically tested to maintain their compliance to written specification. Sampling techniques and laboratory methods should be standardized. Chemical and physical testing will assure that ingredients are uniform, and this will protect the buyer against economic losses. Bacteriological specifications will assure that a product is not a public health risk. At all times, it shall be the responsibility of the supplier to meet the requirements of an ingredient specification.

The manufacturing process. Finished product quality and uniformity are only as good as the process that is used to manufacture such a product. Heavy reliance on process control is very important since production of a large number of units at a high rate makes it virtually impossible to control quality by evaluation of finished product alone. All the para-

meters defining a production process should be standardized. These should be adhered to in manufacturing, and should be changed only if process changes result in a demonstrably improved product.

Usage of in-line control bacteriology as a tool for process control is generally accepted as a better way than just finished product analysis. Assessment of the levels of indicator organisms such as total counts, coliforms, *Escherichia coli* or fecal streptococci throughout the production process is a good way to determine the adequacy of the process and the source of contamination by bacteria.

One has to take into consideration the analytical errors inherent in testing for bacteria besides an appreciation of the usage of indicator organisms as tools in an in-line control program. Many foods such as cheese are normally high in total counts; *E. coli* in cooked frozen foods might indicate contamination after cooking but not necessarily fecal contamination. If equipment was improperly sanitized, *E. coli* could grow in it and contaminate food produced (3). Fecal streptococci can establish themselves in a plant or food far removed from the original source, and complete elimination of these organisms even under good sanitary conditions could be extremely difficult (4). If excessive numbers of indicator organisms are found in line-samples, far above what is considered normal to a production process, one should investigate promptly as to their cause and take immediate remedial action. At all times it might be worthwhile for the food processor to determine levels of indicator organisms associated with his process.

Finished product control. While heavy emphasis should be placed on ingredients and process control, there is need to ascertain that finished products are adequately tested for quality. Flavor evaluation should be conducted on selected samples by trained panelists to find out if the product is acceptable to market. Bacteriological analysis conducted on finished products assures their wholesomeness. The whole problem of sampling for *Salmonella* has been ably discussed in a recent report by the National Academy of Sciences (9).

Environmental sanitation. While sanitation is an important factor in production of wholesome foods, it will not guarantee that the food produced is wholesome. This does not imply that good manufacturing practices should not be practiced. In many instances bacterial contamination is caused by unique environmental situations where the process is inadequate, contamination occurred after processing, or in the ingredients.

The recently published codes of *Good Manufacturing Practices* by the Federal Food and Drug Administration set forth guidelines on good housekeep-

ing practices and production. Written codes have been published by U. S. Public Health Service for the dairy industry and the shell fish industry. Contamination of food with pathogens such as *Salmonella* in foodstuffs at any level is considered a violation of the Federal Food, Drug, and Cosmetic Act and the product is subject to seizure. Much has been said about the "ubiquitous" *Salmonella* organism; in most instances contamination with this organism in cooked foods has been traced to raw materials, inadequate process, or the environment. If these bacteria are in the environment they will eventually find their way into the finished product.

Environmental control is a necessity in the manufacture of cooked frozen foods. Environmental samples to be analyzed could be: air filters, drain residues, in-line sample composites, left-overs, vacuum sweepers, and floor sweepings.

The intelligent food processor will recognize the problem of *Salmonella* contamination and seek to determine where his problem areas are and initiate a standard sampling and testing program using a "flow-sheet" type system.

In most food plants sanitation is associated with housekeeping and cleaning practices. While these two are important, the program should also include maintenance of equipment and pest control. Personal hygiene in food plants should meet the highest standards; operational practices should be clearly defined by management; both supervisory and non-supervisory personnel educated as to the reason behind the rules. Education of production personnel should be conducted on a continuing basis.

Production of foods in facilities which are contaminated with rodents or insects is a violation of the Food, Drug, and Cosmetic Act. Food processors should implement measures to control pests. Dry material such as flour should be sifted through a 30-mesh screen to eliminate larvae and insects. Routine "black light" inspection should be performed on all bagged ingredients to detect rodent urine contamination. All carrier vehicles should be inspected for cleanliness.

Equipment should be properly cleaned and sanitized. The frequency of cleaning and the adequacy of both cleaning and sanitizing should be determined using a bacteriological control program. Generally, swabs of equipment or rinse water analysis are good indicators of cleaning adequacy; Rodac contact plate methods also offer a simple way for determining the same thing.

Maintenance of equipment at scheduled intervals is important. Improperly maintained equipment is conducive to accidents and contamination of product with foreign material. All equipment should be de-

signed for easy access to cleaning. Hard to clean spots can be focal points for bacterial growth. Associations such as Baking Industry Sanitation Standards Committee and International Association of Milk, Food and Environmental Sanitarians have written criteria on equipment design and construction which are useful to the manufacturer.

Hodge (6) reported that 99% of staphylococcal outbreaks he studied were caused by cooked, high protein foods. Left-over foods were responsible for 94% of the outbreaks. These foods had been held unrefrigerated for long periods of time. Strict quality control procedures should be instituted to handle any foods that are left over to be processed further. If at all possible, formulations should be adjusted to prevent generation of excessive amounts of left-overs. Potentially hazardous bacteria do not grow substantially at temperatures below 45 F or above 145 F. Cooked food products must be brought out of the danger range of 45 F to 145 F in less than 3 hr including chill time (1). Time-temperature profiles throughout the production process should take this fact into consideration.

CONCLUSION

Published statistics by the National Communicable Disease Center (14) show where mishandling of food resulted in food poisoning. In 1969, the homemaker was guilty 13% of the time, the commercial eating place 31% of the time, and the food processor 8% of the time. While proper controls by the processor will minimize problems in the field, control will be achieved only if food service personnel are trained in the handling of foods. This problem was put into perspective by the National Academy of Science in a recent report, *An Evaluation of the Salmonella Problem* (9):

"Leadership at the national level is vital to develop a consensus among governmental agencies, industry associations, labor unions, public information media, and school systems regarding the main thrust and timing of educational programs that will complement other actions initiated to minimize the spread of salmonellosis by environmental vehicles . . . the effective control of salmonellosis in the United States depends heavily on developing a continuing nationwide education and training program that will inform, motivate and periodically re-train the multitude of individuals who must help to improve the level of environmental sanitation. Such a program can be effective only when its teachings are put into practice."

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SUPPLEMENT NO. 3 TO THE 3-A SANITARY STANDARDS FOR INSTRUMENT FITTINGS AND CONNECTIONS USED ON MILK AND MILK PRODUCTS EQUIPMENT

Serial #0905

Formulated by

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

This supplement incorporates the following fittings into this standard:

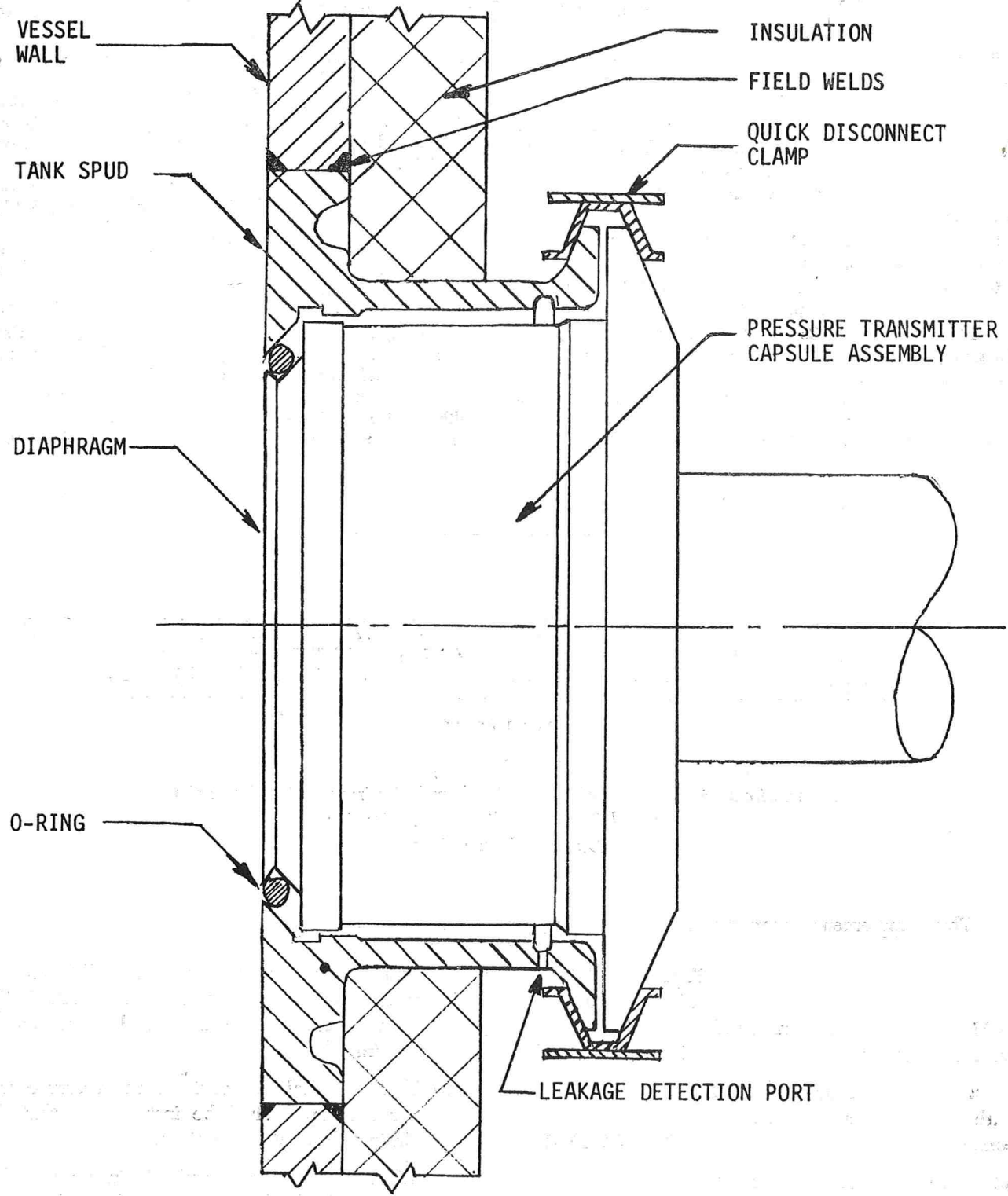
	<i>Page No.</i>	<i>3-A Drawing No.</i>
3A11 Pressure sensor tank spud with O-Ring seal	15	3A-101-13
3A12 Pressure sensor tank spud with gasket seal and bolted connection	16	3A-101-14
3A13 Pressure sensor tank spud with self sealing diaphragm	17	3A-101-15

In addition to the requirements found in section B. Construction of this standard, the fittings incorporated by this supplement shall comply with the following:

- (1) The pressure sensor tank spud shall be welded flush to the inside of the tank (vessel).

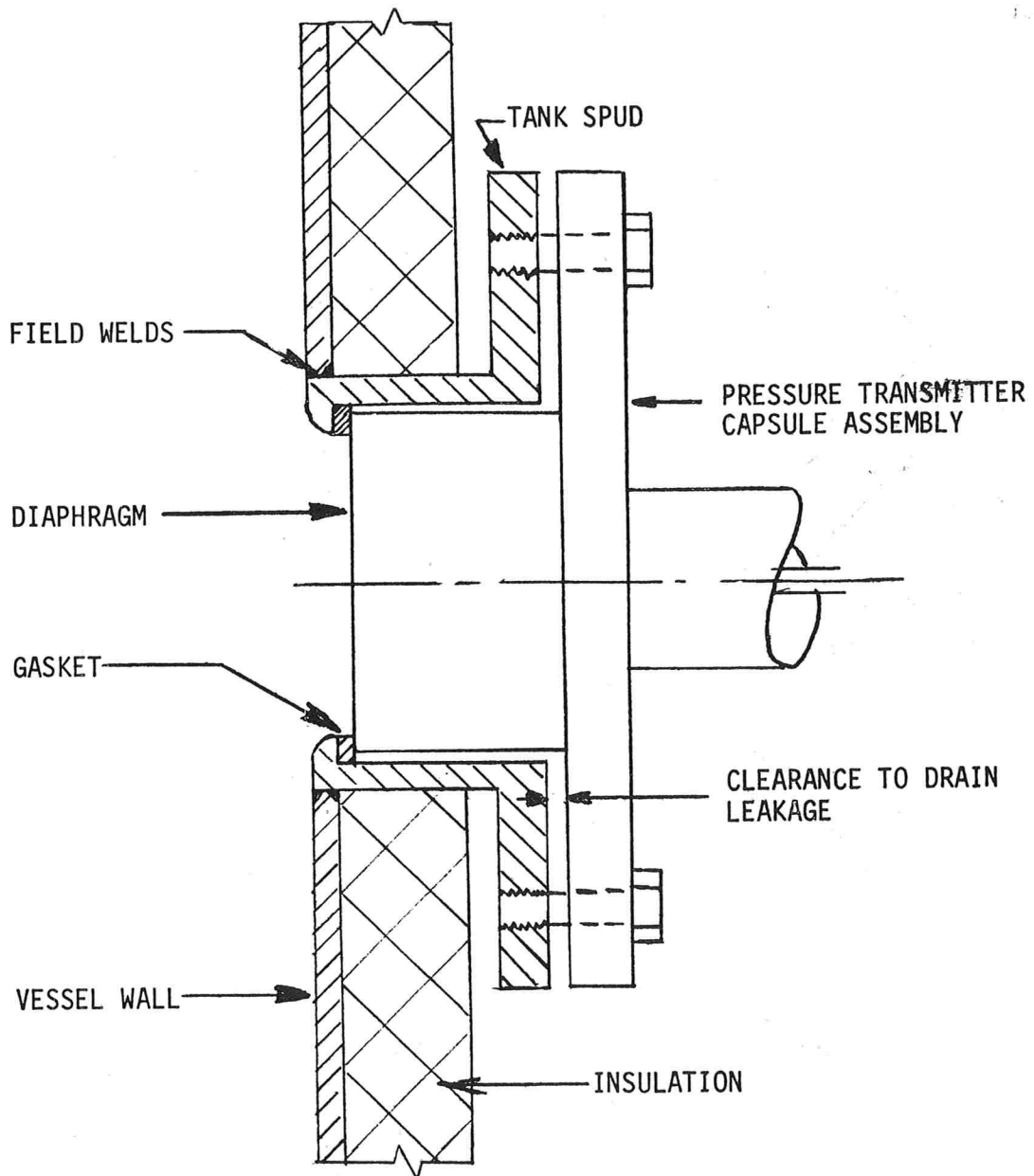
- (2) The pressure sensor tank spud shall have provision to drain leakage of product and if the tank is insulated, leakage shall drain beyond the insulation.
- (3) If a drain hole is provided, the pressure sensor tank spud shall be installed so that the drain hole is at the bottom.
- (4) When the sensor capsule is in its installed position in the level sensor tank spud, the O-Ring or gasket and diaphragm shall form a crevice-free joint.

This supplement is effective May 18, 1972.



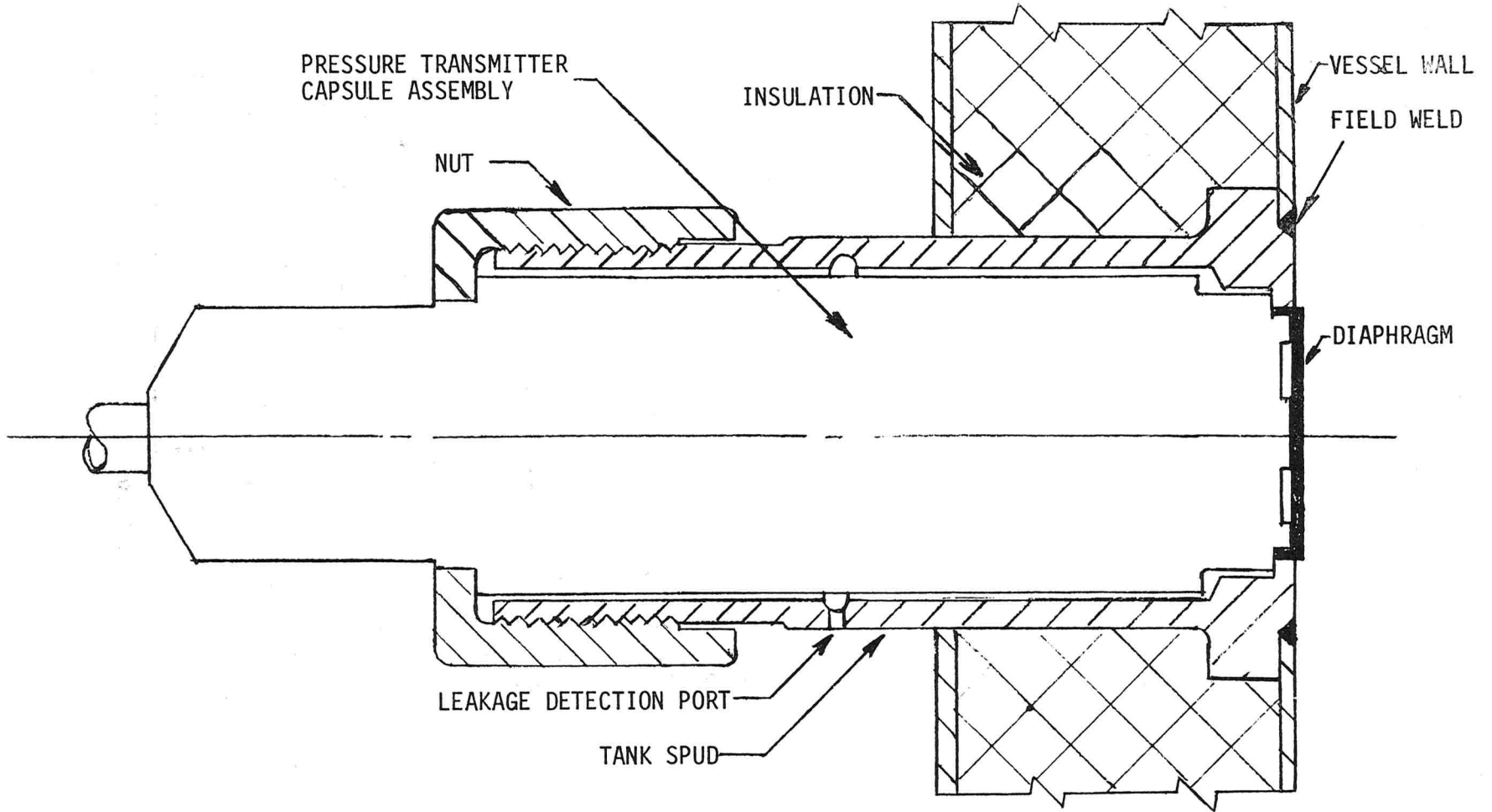
3A11 PRESSURE SENSOR
TANK SPUD WITH O-RING
SEAL

3A STANDARD
INSTRUMENT
FITTINGS & CONNECTIONS
3A-101-13



3A12 PRESSURE SENSOR
TANK SPUD WITH GASKET
SEAL & BOLTED CONNECTION

3A STANDARD
INSTRUMENT
FITTINGS & CONNECTIONS
3A-101-14



3A13 PRESSURE SENSOR TANK
SPUD WITH SELF SEALING
DIAPHRAGM

3A STANDARD
INSTRUMENT
FITTINGS AND CONNECTIONS
3A-101-15

AMENDMENT TO 3-A SANITARY STANDARDS FOR FILLERS AND SEALERS OF SINGLE SERVICE CONTAINERS FOR MILK AND FLUID MILK PRODUCTS

Serial #1703

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

The "3-A Sanitary Standards for Fillers and Sealers of Single Service Containers for Milk and Fluid Milk Products, Serial #1700" are hereby amended in the sections indicated below:

Substitute the following for A. *SCOPE*:

A. *SCOPE*

These standards cover the sanitary aspects of equipment for performing all or a part of the following functions: mechanically opening, filling and sealing single service containers and all parts which are essential to these functions that are furnished by the filler and sealer manufacturer. It does not pertain to other integral equipment embodied on certain machines which perform such functions as container fabricating; nor to the single service container.

In order to conform with these 3-A Sanitary Standards, Fillers and Sealers of Single Service Containers for Milk and Fluid Milk Products shall comply with the following design, material, and fabrication criteria that are applicable.

Substitute the following for definitions B.(2), B.(4), and B.(5) in B. *DEFINITIONS*:

(2) *CONTAINER*: Shall mean a single service package which is to be filled with the product.

(4) *MECHANICAL FILLING EQUIPMENT*: Shall mean the equipment for mechanically filling the container with the product.

(5) *MECHANICAL SEALING EQUIPMENT*: Shall mean the equipment for mechanically closing and/or sealing the filled container.

Add the following definition to B. *DEFINITIONS*:

(7) *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.

Substitute the following for subparagraph (a) of C.(1) in C. *MATERIAL*:

(a) Those surfaces of container opening, closing and sealing devices which touch the product contact surfaces of the container, or from which liquids may drain or drop into the container, may be made of or be plated with a non-toxic, non-absorbent metal that is corrosion resistant under conditions of intended use.

Substitute the following for the first sentence of subsection (b) of section C.(1) in C. *MATERIAL*:

(b) Plastic materials may be used for filling nozzles, plungers, gaskets, diaphragms, sealing rings, short flexible transparent connectors, rollers, belts, drip shields, container opening and closing parts, filling valve members, seals and parts used in similar applications.

Substitute the following for the first sentence of subsection (c) of section C.(1) in C. *MATERIAL*:

(c) Rubber and rubber-like materials may be used for filling nozzles, plungers, gaskets, diaphragms, sealing rings, rollers, belts, drip shields, container opening and closing parts, filling valve members, seals and parts used in similar applications.

Add the following to section D. (3) in D. *FABRICATION*:

Fillers designed to be mechanically cleaned shall be accessible for manual cleaning and inspection.

This amendment is effective May 18, 1972.

INHIBITION OF GROWTH OF AIRBORNE COLIFORMS AND OTHER BACTERIA ON SELECTIVE MEDIA¹

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ABSTRACT

Coliforms when sampled from air were inhibited or restricted in growth on regular selective media. For trials, growth on Standard Plate Count Agar (SPC) was used as 100%. The percentage of colony growth of *Escherichia coli* and *Enterobacter aerogenes* on modified selective media or with SPC were respectively as follows: violet red bile/violet red bile (VRB/VRB) (overlay on base) <4, 15; desoxycholate/desoxycholate (DES/DES) <1, 17; tergitol/tergitol (TER/TER) 23,49; eosine methylene blue/eosine methylene blue (EMB/EMB) 122, 86; endo/endo (END/END) 40, 104; MacConkey/MacConkey (MAC/MAC) <2, 10; SPC/VRB <2, 22; SPC/DES <2, 23; VRB/SPC 54, 60; TER/SPC 72, 119; EMB/SPC 97, 119; END/SPC 95, 90; 1 VRB: 1 SPC, as overlay and base 14, 50. Recovery percentages of *Pseudomonas aeruginosa* and *Pseudomonas fragi* on modified selective media were greater than those of the coliform bacteria. Fewer *Serratia marcescens* colonies grew on DES or SPC/DES and more grew on the other modified selective media than did coliform colonies. Growth of airborne *Salmonella newbrunswick* ranged from <1% on SS agar to 118% on MAC/SPC. Aerosolization of coliforms with skim milk compared to distilled water resulted in growth of more colonies on selective media. Desoxycholate, Bile Salts No. 3, Tergitol No. 7, and crystal violet in the media definitely limited growth of airborne coliforms.

Aerosolized *Escherichia coli* often failed to grow when deposited on desoxycholate agar by means of the Casella sampler. Sunga et al. (16) were unable to recover on violet red bile agar coliform microorganisms shed from arms and hands of dairy plant workers. Adams and Spendlove (1) were able to grow coliforms from the air of sewage treatment plants on eosine methylene blue and endo agars. Speck (13) and Maxcy (9) observed the problem of coliform recovery with selective media after apparent injury from exposure to heat, freezing and thawing, and chemicals such as chlorine.

The extent of injury of airborne coliforms may vary with the exposure time, relative humidity, temperature, composition of the air, means of aerosolization, and method of sampling (3, 6). The toxic effect of water loss in relation to solute concentration on the amount of injury has been reported by several investigators (5, 11, 12). Crystal formation of solute from the loss of water was suggested by Silver (12).

Presence of coliform organisms has been used as an indication of the possibility of the presence of more serious contamination, perhaps from human sources. These trials were initiated to study the recovery of airborne coliforms on selective media with the principal objective of obtaining improved colony growth. Various selective media, modification of the concentration of the inhibitory agents, and their effect on several species or strains were investigated. *Pseudomonas*, *Bacillus*, and *Serratia* species were used in the trials to determine if the medium was in fact selective or if the colonies of these organisms could be distinguished from coliform colonies at various stages of visible growth.

MATERIALS AND METHODS

Escherichia coli strains K12, W1177, and B/r were obtained from the stock cultures in the Microbiology and Public Health or the Food Science and Human Nutrition Departments at Michigan State University. These were grown in nutrient broth at 37 C for 24 hr. Organisms in the stationary phase were used. Suspensions in distilled water of known numbers predetermined by growth on SPC agar were prepared and continuously aerosolized with a glass atomizer (8) at 20 psi of air into an acrylic plastic chamber (2 × 2 × 2 ft). The input of bacteria generally ranged from 10² to 10³ per ft³ of air per minute for solid surface impaction trials and from 10² to 10⁴ per ft³ of air per minute for liquid impingement trials. A small bacterial filter permitted an equilibrium with atmospheric pressure in the chamber. Experiments were performed at room temperature. The relative humidity increased from 75% to 80% after 5 min of aerosolization.

An Andersen sampler (2) was used to sample 1 ft³ of air from the chamber. Bacteria were deposited on the surface of the medium in a petri plate. Each sampling required 1 min. The stage of the sampler with the finest pores was used. The following media were used: violet red bile (VRB), desoxycholate (DES), eosine methylene blue (EMB), MacConkey (MAC), tergitol (TER), endo (END), Standard Plate count (SPC), bismuth sulfite (BS), brilliant green (BG), selenite broth with 1.5% agar (SA), salmonella-shigella (SS) and a mixture of 1 VRB: 1 SPC. Alternate samples were taken onto SPC.

The inhibitory effect of sodium desoxycholate or Bile Salts No. 3 on airborne coliforms was investigated by adding various concentrations, ranging from 0.1 to 2.0 g/l to SPC. The concentration of Tergitol No. 7 varied from 0.01 to 0.1 g/l of SPC and crystal violet was used at the rate of 0.002 g/l of SPC. After the bacteria were deposited on the solidified agar base by the sampler approximately 10 ml of the same medium or SPC (overlay) was used to uniformly cover the

¹Michigan Agricultural Experiment Station No. 5541.

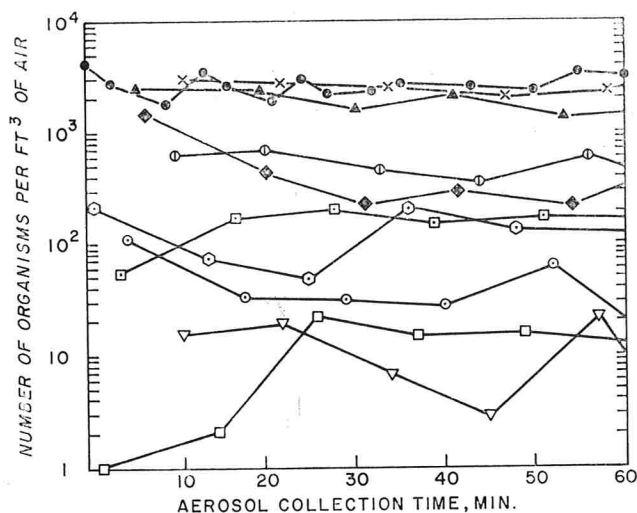


Figure 1. Growth of airborne *Escherichia coli* W1177 aerosolized with distilled water at room temperature and 80% relative humidity on various selective media. Symbols: (x) = TER/SPC (overlay on base); closed triangle = VRB/SPC; closed diamond = 1 VRB: 1 SPC; circle with vertical line = TER; hexagon = VRB; circle with dot = SPC/DES; square with dot = SPC/VRB inverted open triangle = MAC; open square = DES; closed circle = SPC which served as the control.

bacteria and base. Plates were incubated at 32 C for 24 hr and the colonies counted.

The procedure for determining the number of airborne bacteria by liquid impingement consisted of continuously aerosolizing the distilled water suspension of the organisms into the chamber for 10 min. After the first 5 min of continuous aerosolization, an air sample was taken by the glass liquid impinger method (4) for 5 min into Brain Heart Infusion broth with 0.01% silicone antifoam agent (Dow Chemical Co.). Portions of the broth were plated immediately after sampling on SPC, VRB, DES, TER, and 1 VRB: 1 SPC media mixture.

RESULTS AND DISCUSSION

Impaction on agar

Results of growth trials of airborne *E. coli* W1177 on selective media compared to growth on SPC agar are shown in Fig. 1. The least colony growth was obtained on DES and MAC agars, ranging from 1 to 25/ft³. They were approximately 2,000 to 4,000 on SPC or TER/SPC and VRB/SPC.

Cumulative results of growth of several species of aerosolized bacteria are shown in Tables 1 and 2. Recoveries of the various organisms on the media ranged from 159% on EMB overlay and base for *E. coli* B/r to <1% for the three *E. coli* strains on DES overlay and base. Intermediate values were observed for END or TER overlay and the same base, respectively, for the three strains of *E. coli*. The significantly greater recovery of airborne *E. coli* strains on EMB may result from several factors: (a) eosine Y dye in the EMB according to Scheusner et al. (15)

had no apparent inhibitory effect on *E. coli* injured by a quaternary ammonium compound: (b) EMB does not contain bile salts which are inhibitory for organisms already injured (15); and (c) the lactose and/or saccharose present in EMB may have promoted recovery of airborne coliforms to a greater degree than on SPC. Considerable improvement was observed (in comparison to using the selective media only) when the organisms were impacted on SPC plus an overlay of TER, VRB, or END. Only moderate improvement was observed when the concentration of inhibitory agents was reduced one-half by diluting the VRB with an equal volume of SPC pouring the base and overlay.

Of the above two alternatives, SPC base or dilution of the medium with SPC to reduce toxicity of the selective medium for airborne coliforms, use of SPC as a base with subsequent (1-1 1/2 hr delay after impaction) overlay with the selective medium was generally the more successful (Table 1). The probable reason is that during the time elapsing between impaction and overlaying the organisms injured by the airborne state had recovered to some extent in the SPC before they were covered with the selective medium.

Attempts to reduce inhibition of growth by diluting VRB with an equal volume of SPC was not as successful as impaction on SPC and overlay with VRB. Growth of various strains of airborne coliform organisms was decreased by addition of Bile Salts No. 3 (Difco) to SPC (Fig. 2). As the concentration of Bile Salts was increased, growth of airborne coliforms began to show an increasing inhibition with concentrations much below the 1.5 g/l used in the selective media. Crystal violet and neutral red may cause reduction of growth as these dyes have been shown to be inhibitory for chemically injured *E. coli* ML30 (15).

The inhibitory effect on airborne *E. coli* strains (K12 and W1177) was in direct relationship to the increasing concentration of desoxycholate as shown by Fig. 3. This figure also indicates improved colony growth by using SPC base as compared to overlay and base with 0.1 to 1.0 g/l sodium desoxycholate. Growth of *E. aerogenes* changed little when only the overlay contained desoxycholate with increasing amounts up to 1 g per liter of SPC. Scheusner et al. (15) reported that the growth of *E. coli* ML30, after injury with a quaternary ammonium compound, was considerably reduced by bile salts and especially by sodium desoxycholate. Likewise, a similar effect was observed with airborne coliforms. The specific nature of the injury may not necessarily be the same in both instances.

Growth of airborne *E. aerogenes* was usually

TABLE I. GROWTH OF AEROSOLIZED BACTERIA ON SELECTIVE OR MODIFIED COLIFORM MEDIA

Species	No. of samples	Organisms per ft ³ air	Media								
			SPC/SPC	VRB/VRB	DES/DES	SPC/VRB	SPC/DES	VRB/SPC	1 VRB:1 SPC	TER/TER	TER/SPC
<i>E. coli</i> K12	52	Range	598-6800	0-150	0-28	0-86	0-68	162-3840	15-880	29-2836	472-5370
		Average	2481	32	4	18	16	1104	154	621	1960
		%	100	1.3	0.2	0.7	0.6	44	6.0	25	79
<i>E. coli</i> B/r	48	Range	26-736	0-17	0-3	0-23	0-20	5-352	0-196	0-320	13-136
		Average	154	2	0.4	3	2	60	28	38	66
		%	100	1.3	0.3	1.9	1.3	39	18	25	43
<i>E. coli</i> W1177	32	Range	610-4700	41-640	1-130	18-295	3-88	941-3170	210-970	210-1900	1290-4000
		Average	2625	193	17	41	32	2074	465	528	2439
		%	100	7.4	0.6	1.6	1.2	79	18	20	93
<i>E. aerogenes</i>	24	Range	346-9400	68-2200	6-2100	58-2900	58-2300	400-5400	112-6100	169-4600	410-790
		Average	3052	457	518	682	715	1840	1523	1495	3630
		%	100	15	17	22	23	60	50	49	119
<i>P. fragi</i>	14	Range	222-3200	620-3248	59-564	264-2248	9-234	490-3132	—	944-3584	448-2000
		Average	1350	1533	326	1198	121	1347		1468	1308
		%	100	114	24	89	9	100		109	97
<i>P. aeruginosa</i>	18	Range	90-2208	186-1888	80-1048	352-1536	48-432	348-1296	86-2140	8-1664	3-912
		Average	824	885	426	231	231	677	887	472	453
		%	100	107	52	28	28	82	108	57	55
<i>S. marcescens</i>	18	Range	568-11,330	258-2944	45-228	294-1468	9-268	296-12,064	496-8352	208-9760	606-9856
		Average	5194	1620	109	683	155	4429	2472	4524	4622
		%	100	31	2	13	3	85	48	87	89
<i>B. subtilis</i> 5320 spores	4	Range	590-840	<1	<1	<1	<1	<1	<1	<1	0-88
		Average	663	<1	<1	<1	<1	<1	<1	<1	38
		%	100	<1	<1	<1	<1	<1	<1	<1	6

greater in the various concentrations of Bile Salts No. 3 and sodium desoxycholate than growth of the various strains of *E. coli* (Fig. 2 and 3). The detrimental effect of Bile Salts No. 3 and sodium desoxycholate on *E. aerogenes* was approximately the same but desoxycholate was more inhibitory to *E. coli* than Bile Salts No. 3. Growth of aerosolized *E. coli* K12 on SPC containing 0.002 g/l of crystal violet was reduced to 67%. Tergitol No. 7, used at the rate of 0.01-0.1 g/l of SPC, reduced growth of airborne *E. coli* K12 from an average of 40 to 3.5%

The effects of skim milk as an aerosolization medium on growth of three strains of airborne *E. coli* are shown in Table 3. A comparison of growth percentages is presented in Tables 1 and 3. Table 3 indicates that on all of the selective media studied, recovery of these strains improved when the organisms were aerosolized from skimmilk instead of distilled water. These results indicate that the skimmilk provided more protection against injury caused by the airborne conditions than distilled water. Stersky (14) reported similar results in a study that involved aerosolization of *Salmonella newbrunswick* with water and with skim milk.

In commercial food plants recovery of airborne coliforms on selective media probably would be difficult. But, if the air contained organic material, as is common in some areas of a milk drying plant, limited growth could occur on the selective medium. Recovery is influenced by the amount and kinds of organic or other materials which might be present in the environment in which the coliforms become airborne. A large number of organic compounds have been reputed to be protective for airborne microorganisms (17).

Growth of aerosolized *E. aerogenes*, as shown by Tables 1 and 2, was greater than for the *E. coli* strains on VRB, DES, TER, END, MAC, SPC/VRB, 1 VRB: 1 SPC, TER/SPC, MAC/SPC, and SPC/DES. However, on EMB, VRB/SPC and EMB/SPC airborne *E. aerogenes* and the three strains of *E. coli* showed comparable growth. The relatively greater growth of *E. aerogenes* on some inhibitory media compared to recovery of the *E. coli* strains may result from greater natural resistance of this species.

Table 1 indicates that other airborne gram-negative organisms (*Pseudomonas fragi*, *Pseudomonas aeruginosa*, and *Serratia marcescens*) grew on selective media used for coliforms. In fact, *P. fragi* and *P. aeruginosa* grew well on VRB/VRB and *S. marcescens* on TER/TER. Nevertheless, the colony morphology (e.g. size and color of colony, discoloration of the surrounding media) of these organisms was distinctly different from that of the coliforms. But, *S. marcescens* appeared as red colonies on VRB.

TABLE 2. GROWTH OF AEROSOLIZED COLIFORMS AND *S. Newbrunswick* ON VARIOUS SELECTIVE MEDIA

Species	No. of samples	Organisms per 10 ⁶ air	Media															
			SPC/SPC ¹	EMB/SPC	EMB/EMB	SPC/SPC ¹	END/SPC	END/END	SPC/SPC ¹	MAC/SPC	MAC/MAC	SS/SS	SA/SPC	SS/SA	MAC/SPC	BS/BS	BG/SPC	BG/BG
<i>E. coli</i> K12	20	Range	354-3056	398-1232	232-4528	2-356	3-162	0-61	62-1092	35-818	<1							
		Average %	1203	733	1319	39	42	11	300	183	<1							
<i>E. coli</i> B/r	20	Range	32-328	41-442	21-608	5-724	2-388	1-400	14-344	10-136	<1							
		Average %	151	192	240	173	148	128	82	50	<1							
<i>E. coli</i> W1177	20	Range	52-452	56-588	53-465	6-2256	5-2208	0-564	736-7072	508-2640	2-86							
		Average %	163	170	160	330	301	60	2139	1118	26							
<i>E. aerogenes</i>	20	Range	872-2240	662-4208	590-3152	155-2600	185-3584	314-2736	195-1768	205-1552	11-133							
		Average %	1410	1684	1211	1056	950	1098	507	543	50							
<i>S. newbrunswick</i>	20	Range	400-2163	204-1190	<1	420-2200	<1	472-2550	40-230	300-1800	160-850							
		Average %	978	509	<1	1076	<1	1154	127	782	362							

Each of these three columns of SPC/SPC data were obtained with different conditions, e.g. cultures, technicians, etc.

As indicated by results in Table 1, the *Pseudomonas* species and *S. marcescens* showed more colonies on the various selective media than the three strains of *E. coli*. However, *S. marcescens* counts on DES or SPC/DES were low.

Growth of *Bacillus subtilis* (Table 1) was inhibited even when the concentration of inhibitory substances was reduced either by using lower concentration (1 VRB: 1 SPC) or by using SPC as a base with 1 VRB: 1 SPC as an overlay.

Results of growth of airborne *S. newbrunswick* on salmonella-shigella Agar, bismuth-sulfite, brilliant green, and selenite agars with or without SPC base are shown in Table 2. Percentages of growth of these airborne organisms ranged from less than 1% on SS and SA to 118% on MAC/SPC or 110% on SA/SPC. These results indicate that aerosolization adversely affects growth of *S. newbrunswick* on selective media for salmonellae.

The probable effects of bile salts on the injured bacteria can be derived from the literature. Hill (7) used aqueous solutions of bile salts for the "extraction" of theicic acid and polysaccharides from the cell walls. McIntire et al. (10) reported "in vitro" disaggregation of subunits of a lipopolysaccharide isolated from *E. coli*.

On the basis of these findings, it is conceivable that organisms which have sustained injury in the airborne state become further debilitated by bile salts thus weakening their cell walls. This injury to the cell wall may lead to further deterioration of permeability of the cell membrane which may already be damaged while in the airborne state and subsequently exposed to adverse osmotic conditions. Loss of cell membrane permeability in airborne bacteria has been reported (4, 14).

Liquid impingement results

Results presented in Table 4 indicate that growth of two strains of *E. coli* aerosolized with distilled water, sampled by liquid impingement, and plated on various media gave slightly greater growth on VRB (4.0% compared to 1.3%) than was obtained by solid surface impaction sampling (Table 1). However, the difference was not statistically significant. Growth was 69% for *E. coli* K12 on TER (Table 4) by liquid impingement method which was greater than on the solid surface impaction agar (25% for *E. coli* K12, Table 1) but the reverse occurred with strain B/r (6 and 25%). Direct or indirect results on DES and 1 VRB: 1 SPC were comparable to those obtained by the Andersen sampler (Tables 1 and 4).

As shown by results in Table 5 the percentage growth of *E. coli* strains aerosolized with skim milk and then sampled by liquid impingement and plated

TABLE 3. GROWTH OF *Escherichia coli* ON SELECTIVE MEDIA AFTER AEROSOLIZING WITH SKIM MILK

Strain	No. of samples	Organisms per ft ³ air	Media									
			SPC/SPC	VRB/VRB	DES/DES	SPC/VRB	SPC/DES	VRB/SPC	1 VRB: 1 SPC	TER/TER	TER/SPC	
<i>E. coli</i> K12	43	Range	124-3616	84-1744	0-960	148-1744	53-1364	148-3248	84-2384	156-2698	124-3440	
		Average	580	350	211	408	275	584	502	514	659	
		%	100	60	36	70	47	101	87	89	114	
<i>E. coli</i> B/r	36	Range	31-388	14-480	6-464	16-328	11-400	41-436	30-544	37-342	30-362	
		Average	155	129	55	112	84	142	145	114	139	
		%	100	83	35	72	54	92	94	74	90	
<i>E. coli</i> W1177	36	Range	116-2544	22-1200	90-1760	40-1952	72-1552	133-2112	79-2032	120-1792	154-2016	
		Average	832	336	556	382	435	703	469	520	643	
		%	100	40	67	46	52	84	56	62	77	

TABLE 4. GROWTH OF *Escherichia coli* AEROSOLIZED WITH WATER AND SAMPLED BY LIQUID IMPINGEMENT METHOD

Strain	No. of samples	Organisms per ft ³ air	Media				
			SPC	VRB	DES	1 VRB:1 SPC	TER
<i>E. coli</i> B/r	14	Range	1720-15,700	51-688	3-41	290-2660	1320-8020
		Average	4000	176	13	654	250
		%	100	4	0.3	16	6
<i>E. coli</i> K12	18	Range	4300-12,400	93-980	10-109	340-2510	2080-7520
		Average	6353	283	48	967	4382
		%	100	4	0.8	15	69

TABLE 5. GROWTH OF *Escherichia coli* AEROSOLIZED WITH SKIM MILK AND SAMPLED BY THE LIQUID IMPINGEMENT METHOD

Strain	No. of samples	Organisms per ft ³ air	Media				
			SPC	VRB	DES	1 VRB:1 SPC	TER
<i>E. coli</i> B/r	14	Range	20-80	17-198	3-69	57-640	97-800
		Average	308	59	25	198	271
		%	100	19	8	64	88
<i>E. coli</i> K12	20	Range	380-1550	40-572	21-468	149-944	356-1460
		Average	871	229	176	476	756
		%	100	26	20	55	87
<i>E. coli</i> W1177	10	Range	80-123	18-62	9-38	33-70	43-61
		Average	97	41	19	46	53
		%	100	42	20	47	55

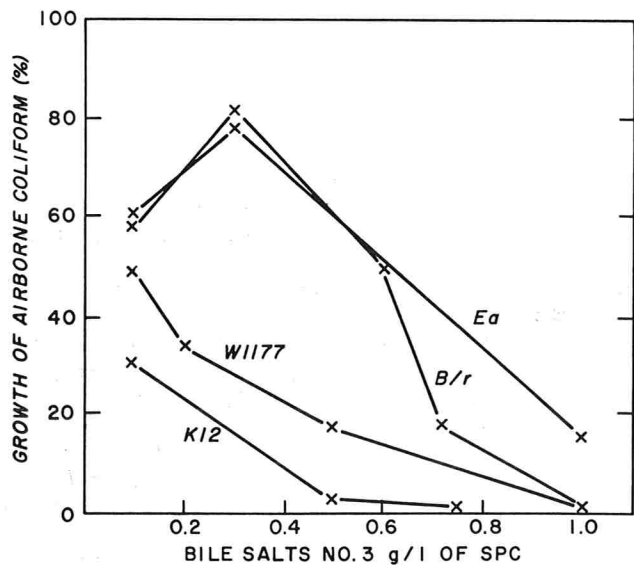


Figure 2. The effect of Bile Salts No. 3 concentration in Standard Plate Count Agar on the growth of airborne coliforms. Percent growth is based upon airborne growth on Standard Plate Count Agar as 100%. Symbols: Ea, *Enterobacter aerogenes*; B/r, W1177, K12, strains of *Escherichia coli*.

on selective media was greater than when the organisms were aerosolized from distilled water (Table 4). However, the percent growth on selective media after liquid impingement, in most trials, was lower (Tables 5 and 3) than when the sampling was done by the Andersen sampler.

CONCLUSIONS

Growth of airborne coliforms and *S. newbrunswick* is reduced or inhibited by selective media when compared to growth on SPC. A larger number of colonies may be obtained by use of a modified selective medium having a reduced dye content by the addition of SPC or SPC base with a selective med-

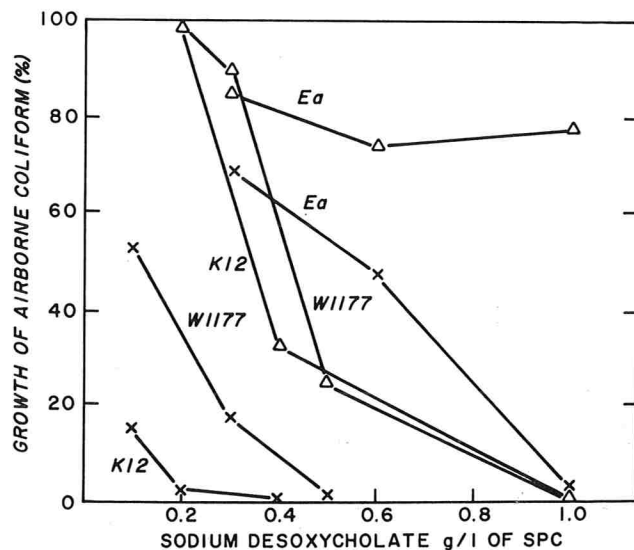


Figure 3. The effect of sodium desoxycholate concentration in Standard Plate Count Agar on the growth of airborne coliforms. Symbols: Ea = *Enterobacter aerogenes*; W1177 and K12 = strains of *Escherichia coli*; open triangles = only the overlay contained desoxycholate; (x) = both overlay and base contained desoxycholate.

ium overlay. Some non-coliforms such as *P. fragi*, *P. aeruginosa*, and *S. marcescens* may grow on the modified medium but these colonies are visually distinguishable from coliform colonies.

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REPORT OF THE EXECUTIVE SECRETARY AND MANAGING EDITOR, 1970-1971

On July 1, 1971 I completed 20 years as your Executive Secretary and I am happy to report it was the biggest year we have ever had with respect to total income. The income was over \$61,000 which exceeds the previous high by \$4,000. Unfortunately it was not the biggest from the standpoint of net income. We are, of course, the victim of constantly rising prices which made it impossible to increase our net income even though our gross income was higher. However, the higher income did enable us to show a profit.

We are faced with a dilemma this year, however, since as of this August (1971) we will have a 20% increase in the cost of Journal publication; a 20% increase in postage costs began in May. Since our dues are on a calendar year basis, our subscriptions are on an annual basis with most beginning January 1, and our advertising is on a 3, 6, and 12 time per year basis, we will not be able to realize any increased income until January 1972. This means that we will be faced with approximately six months of increased cost with no way to meet it. We have increased the subscription rate, reprint prices, and advertising rates. It will be up to the Executive Board if there is to be any increase in dues. I believe we should increase our annual dues by \$1.00 or \$2.00. We would still be considerably lower than any other organization of our kind. If we experience no appreciable drop off in these areas, we should be able to improve our situation. I am anxious to

improve our position in order to attract a competent person to replace me on retirement. If the present situation continues, I am sure it will require more funds for clerical help and to fill my position than is now being expended. We can make good progress in this matter in the next two years if we act now.

Elmer Marth will report to you on the *Journal*, but this will be the biggest volume ever published so I sincerely believe it is a bargain and will continue to be even with the increase in price.

Our membership, both affiliate and direct, remains approximately the same. Our subscriptions to the *Journal* again showed an increase and continues to average over 4,000 copies per month.

Thus for the 20th time it has been a privilege and a pleasure to serve you.

Respectfully submitted,

H. L. THOMASSON
Executive Secretary and
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FACTORS IN SURVIVAL OF *CLOSTRIDIUM BOTULINUM* TYPE E SPORES THROUGH THE FISH SMOKING PROCESS

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ABSTRACT

Fish, experimentally inoculated with 1×10^6 *Clostridium botulinum* type E spores, were given heat treatments equivalent to those used in commercial smoking (180 F for 30 min following come-up time of 2.5 - 3 hr). The percentages of such fish containing viable type E spores were significantly lower among fish heated in an environment of high moisture than among those heated in atmospheres of low moisture. Type E spores heated in raw fish mince were more heat resistant than those heated in mince that was previously autoclaved. A similar difference was observed for spores in raw and cooked egg white, another substrate which coagulates rapidly at the temperature of thermal death time studies (82.5 C). Spores in the drippings of fish hung for heating survived heat processing and could be a source of cross contamination.

The low heat resistance of *Clostridium botulinum* type E spores is shown by the D_{176} range of 0.33 to 3.3 when they are heated in phosphate buffer, water, and broth (5, 6, 7). In previously autoclaved fish mince, the most resistant spore suspension derived from five different strains had a D_{176} value of 4.3 (3).

Contrary to the prediction from the above data, an unsuspected resistance was shown by type E spores during the smoking of fish. Viable spores were detected in most fish which were inoculated in the loin muscle with 1×10^6 spores and then smoked for 30 min at an internal temperature of 180 F (2). These spores did not, however, germinate and produce toxin if the fish had brine levels of 3% or higher.

A possible explanation of the anomalous result was based on the moisture of the environments in which the spores were heated (1, 2). Usual laboratory heat resistance studies are performed with a closed system of high moisture while smoking of fish is done in an essentially open system with less sporocidal dry heat. Thus, in smoke processing of fish, dry heat is being applied to the type E spores that settle on the surface of fish when part of the intramuscularly injected spore inoculum leaks out. However, the majority of the experimentally spore-inoculated fish had viable type E spores when processing in the smoke chamber was done with steam (2).

The present communication presents data which show that survival of type E spores through the fish smoking process is influenced by the moisture levels of the heating environments. Consideration is given to another factor which contributes to the heat re-

sistance of the spores that are deposited in the muscles of fish.

MATERIALS AND METHODS

Culture medium (TPGY), spore suspensions, and the method for studying survival of type E spores through the smoke processing of fish (chub: *Leucichthys hoyi*) have been previously described (2). The test was simplified by not using smoke during heating since interpretation of results would not be influenced by its absence. Inocula of 0.1 ml (1×10^6 spores, Alaska E43 or Minnesota strain of type E) per fish were injected intramuscularly or spread over the outer surface. "Smoking" consisted of heating unbrined fish for 30 min at an internal temperature of 180 F (82.5 C) after a come-up time to processing temperature of 2.5 to 3.0 hr.

A single fish (tail removed) was hung by strings inside each 2-quart Mason jar. For heating at high moisture level, 200 ml of TPGY was added, with the fish suspended above and not touching the broth. These jars were partially closed by using caps in which a 6-mm diameter hole had been punched. For low moisture level heating, broth was omitted and the jars were left uncovered. Jars were placed randomly in the smoking chamber so that fish could be heated concurrently at high and low moistures in the same trial. Chances of cross contamination were reduced with the fish inside the jars.

Temperatures were monitored by recording through thermocouples that were inserted into the loin muscle of representative fish. After heating, fish were lowered to the bottom of the jars and 200 ml TPGY was added to those jars not already having the broth. All jars were closed with intact screw caps and incubated 1 week at 25 C. Development of type E toxin during the incubation indicated survival of type E spores through the heating process (2). Previous work showed that detection of survivors was not improved significantly by longer incubations of 2 to 3 weeks.

Fish mince for thermal death time (TDT) experiments was prepared with chub loin muscles dissected out after the skin of the fish had been removed. The flesh was put through a meat grinder and comminuted further by briefly grinding with a mortar and pestle. Applicable aseptic precautions were used. Storage was at -20 C for not more than 1 week.

Thermal death time (TDT) determinations were done in 15×85 mm pyrex test tubes. The stored mince was mixed after thawing and 2-g amounts gently tamped down into the bottom of each sterile, cotton-plugged tube. Some of the tubes were autoclaved (121 C for 15 min). Spores (1×10^6 in 0.1 ml) were deposited near the center of the tubed minces (raw and autoclaved) with a hypodermic needle and syringe. Cotton plugs were replaced with water tight serum bottle stoppers.

The two sets of tubes were heated simultaneously in a water bath at 82.5 ± 1 C while completely submerged and in an upright position. Boiling water was added to the bath to correct the temperature drop that followed immersion of

TABLE 1. SURVIVAL OF *C. botulinum* TYPE E SPORES WHEN FISH, INOCULATED WITH 1×10^6 SPORES, WERE HEATED (180 F FOR 30 MIN) IN ATMOSPHERES OF LOW AND HIGH MOISTURE LEVELS¹.

Moisture ²	Spore inoculum	Fish with viable type E
		Number fish tested
High	Surface	4/61
"	Nil (control)	1/40
Low	Surface	15/40
"	Nil (control)	1/20
High	Intramuscular	35/36
"	Nil (control)	0/35

¹Consolidated results of several trials performed with spores of Alaska E43 and Minnesota strains.

²High moisture: fish heated in semiclosed jars while suspended over broth; low moisture: fish heated while suspended in uncovered jars not containing broth.

TABLE 2. HEAT RESISTANCE OF TYPE E SPORES (1×10^6 /TUBE) HEATED (82.5 C) IN RAW AND PREVIOUSLY AUTOCLAVED (121 C, 15 MIN) FISH MINCES.

Minutes heating	Number with type E survivors/6 tubes tested											
	Minnesota strain spores				Alaska E43 strain spores							
	Autoclaved		Raw		Autoclaved		Raw					
8, 11, 14,	3,	2,	1,	—,	—,	—,	6,	6,	5,	—,	—,	—,
17, 20, 23,	0,	0,	0,	—,	—,	—,	4,	2,	0,	—,	—,	6,
26, 29, 32,	0,	— [*] ,	—,	—,	6,	5,	0,	0,	0,	6,	6,	6,
35, 38, 41,	—,	—,	—,	6,	6,	6,	—,	—,	—,	4,	6,	6,
44	—			4			—			6		

*—: not tested

the large number of tubes. Temperature correction took about 2 min; timing for the TDT was started 1 min later. At selected heating intervals, six tubes of a set were removed and cooled rapidly by transferring to an ice-water bath. TPGY (3 ml) was then injected aseptically into all tubes through the rubber diaphragms. After shaking, tubes were incubated 2 weeks at 25 C at which time the supernatant fluid was tested for type E toxin.

RESULTS

Viable type E spores were present in only a low percentage of fish surface-inoculated with spores and heated (equivalent to smoke processing) in an environment which would have high moisture during the heating (Table 1). In contrast, a significantly higher percentage of similarly inoculated fish had type E spore survivors when heated in an atmosphere of low moisture. That the two sets of fish were heated in different moisture levels was shown by the "mushy" appearance of fish heated in jars containing broth and the dry appearance of those heated in jars not having a moisture source.

When high moisture conditions prevailed, the percentage of fish with type E survivors was significantly higher among chub given an intramuscular spore inoculum than among fish inoculated on the surface with the same number of spores. A possible ex-

planation could be the actual spore loads carried by the two groups of fish; more spores are probably lost in the drippings from fish after surface inoculation than after intramuscular injection. However, there was the possibility that the location of the spores could be a factor.

Spores which are retained intramuscularly after injection are, at the start of heating, in a milieu of raw muscle. This consideration led to a comparison of the heat resistances of type E spores placed in raw and autoclaved fish minces. In the five tests made, spores heated in raw mince were more resistant than those inoculated into heated mince. Two representative test results are given in Table 2.

The observed difference was not because raw mince had a high natural type E contamination which would be absent from pre-heated mince. Each test included 6 to 10 control tubes of raw mince (not experimentally inoculated with type E spores) which were heated 2 or 5 min at 82.5 C. When type E toxin developed in any of these controls, the experimental results were discarded as unreliable because the raw mince could have had a significantly higher initial number of type E spores than the precooked mince.

Type E spores (1×10^6) in 2.5 ml of TPGY were heated in TDT tubes. Six tubes were removed at intervals during heating at 82.5 C. One milliliter from each heated tube was transferred into a tube containing 2 g of raw fish mince ("pasteurized" at 82.5 C for 15 min) plus 2 ml TPGY and into another tube with the same amount of autoclaved mince plus broth. The two recovery media were equally effective in detecting survivors in the heated spore suspensions (Table 3).

The experiment comparing survival of spores in raw and cooked fish mince was repeated using fresh egg white obtained from a commercial egg processor. Two of four egg white samples proved unsuitable in the sense that 5 min heating in a 82 C water bath did not cause firm coagulation of the egg white (20% egg white in water, v/v; 3 ml in 15×85 mm TDT tubes). The experimental procedure was identical to that used with fish mince except that 3 ml of the diluted egg white was used in each tube and spores were inoculated into heated (100 C for 10 min) egg white after the coagulum was broken with a glass rod. Results with spores of the Alaska E43 strain (Table 4) were essentially the same as those with the Minnesota strain; i.e., spores in the raw product were more heat resistant than those added to the product that had been heated previously.

Moisture drips from fish hung in the smoking chamber preparatory to heating. The fate of spores in these drippings was examined. Fish were inocu-

TABLE 3. COMPARISON OF RECOVERY MEDIA CONTAINING RAW OR AUTOCLAVED FISH MINCE FOR DETECTION OF VIABLE TYPE E SPORES IN A SUSPENSION¹ HEATED (82.5 C) FOR DIFFERENT TIMES.

Minutes heating	Number with viable type E/Number tubes heated	
	Raw mince	Autoclaved mince
3	4/6	6/6
6	5/6	6/6
9	1/6	1/6
12, 15, 18	0/6, 0/6, 0/6	0/6, 0/6, 0/6

¹Total 1×10^6 spores in 2.5 ml of TPGY; after heating, contents of each tube subcultured, 1 ml in raw fish medium and 1 ml in autoclaved fish medium.

TABLE 4. HEAT RESISTANCE OF TYPE E SPORES (1×10^6 /TUBE) HEATED (82.5 C) IN 3 ML OF RAW AND PRE-COAGULATED (10 MIN AT 100 C) EGG WHITE.

Minutes heating	Number of tubes with viable type E/6 tubes tested	
	Raw	Precoagulated
5, 10, 15,	—*, —, —,	6, 0, 0,
30, 40, 50,	6, 5, 6,	0, 0, —,
60, 70, 80,	5, 4, 1,	
90, 100	0, 0	

*Not tested

TABLE 5. SURVIVAL OF *C. botulinum* TYPE E SPORES IN THE DRIPPINGS¹ COLLECTED FROM CHUB INOCULATED INTRAMUSCULARLY WITH 1×10^6 SPORES. DRIPPINGS HEATED IN JARS WITH OR WITHOUT TPGY DURING "SMOKING" (180 F, 30 MIN) OF FISH.

Specimen	Heating moisture ¹	Number with viable type E
		Number tested
Fish	low	32/32
Drrippings	low	14/16
Drrippings	high	0/16

¹Low: drippings heated in open jars without broth; high: drippings heated in partially closed jars with broth; fish, from which drippings obtained, heated without jars.

lated intramuscularly with 1×10^6 spores and were hung in the smoking chamber so that the drippings from individual fish could be collected in separate Mason jars. After 10 min, jars were moved from beneath the fish. TPGY (200 ml) was added to one-half of the collections. These jars were covered with lids having a hole; jars to which broth was not added were left uncovered. The chamber was then operated as for the "smoking" of fish. Processed fish were placed in jars and 200 ml of broth added to all jars not already containing it. Presence of toxin after 2 weeks incubation at 25 C was used as an index of spore survival (Table 5). Viable type E spores were found in most of the drippings collected and heated in a dry atmosphere while they were absent from the drippings heated in broth.

Glass funnels were used to collect into test tubes

(15×125 mm) drippings from 25 other fish inoculated intramuscularly with 1×10^6 type E spores. After approximately 3 ml of the initial (immediately after hanging) drippings were collected from each fish, the tubes were closed with serum bottle stoppers and were heated 30 min while submerged in a 82.5 C water bath. The heated tubes were incubated after receiving 3 ml of double strength TPGY. Type E toxin did not develop in any of these tubes.

DISCUSSION

Drrippings, collected from fish experimentally inoculated with type E spores, when heated at 180 F for 30 min, contained survivors of the inoculum provided the heating conditions permitted desiccation. When drying was not possible (semi-closed jars with broth or closed test tubes), survivors were not found. These observations agree with the results obtained when fish itself was heated in atmospheres of low and high moisture in the present work and with the observations of Pace (cited in ref. 1). Thus, one factor that favors survival of type E spores through the smoke processing of experimentally spore-inoculated fish is drying of the spores on the surface of fish during application of dry heat.

The results also suggest a source of cross contamination during smoking of fish. As drippings with spores dry during the early stages of smoking, spores could become air-borne and be circulated in the smoke chamber. Should they lodge on fish, either during or after the heat processing, the fish would have viable type E spores. The significant number of sterilized (121 C for 30 min) fish which had viable type E spores when smoked together with experimentally inoculated fish (2) could be explained on this basis.

Spores heated in raw fish mince had significantly greater heat resistance than those heated in pre-cooked fish mince. The same general finding was duplicated using egg white, another substance that coagulates rapidly at the temperature used to heat the spores. With the fish substrate, the difference was shown not to result from a heat labile factor in raw fish which stimulates outgrowth of heat injured spores. The implication is that the physical state of the suspending menstruum is important in the heat tolerance of the spores.

Type E spores, like spores of other species, have least heat resistance when heated in water activity of 1.0; only a slightly lower water activity increases the resistance by 10 or more fold (4). Results with spores heated in raw and pre-cooked menstrua could result because, at the time of heating when spores have not all been killed, the proteins in the raw substrate coagulate and coat the spores. The same process

could not occur when a prior heating has already coagulated the proteins. The coating could result in the spores being in a microenvironment of low water activity in which they are better able to withstand heat.

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PUBLICATIONS RECOMMENDED BY THE DAIRY FARM METHODS COMMITTEE

Identification	Title	Write to:
Special Report Manual - 29 (no cost)	<i>Dairy Plant Fieldman, Hauler, Grader, and Tester's Manual</i>	Agriculture Extension Service, University of Minnesota, St. Paul, Minnesota
Circular 347 (no cost)	<i>The Quality Milk and Mastitis Program</i>	Florida Cooper- ative Extension Service, Institute of Food and Agri- culture Sciences, U. of Florida, Gainesville, Florida
Special Circular 134 (no cost)	<i>Sampling Milk for Milk Fat Testing</i>	Pennsylvania State University, College of Agriculture Extension Service, University Park, Pennsylvania
Bulletin 388 (no cost)	<i>Milk Composition—How It Affects the Dairyman and How It Varies</i>	University of Kentucky, College of Agriculture Extension Service, Lexington, Kentucky
Special Publication, No number (no cost)	<i>Disposal of Liquid Wastes From Parlors And Milkhouses</i>	Pennsylvania State University, College of Agriculture Extension Service, University Park, Pennsylvania
Publication 1404 (no cost)	<i>Ventilation of Livestock Buildings</i>	Canada Department of Agriculture, Information Division, Ottawa, Canada

BACTERIOLOGICAL EXAMINATION OF RAW BREADED FROZEN SHRIMP¹

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ABSTRACT

Total plate counts and tests for enterococci, total coliforms, and *Escherichia coli* were made on a number of frozen raw, breaded shrimp samples. These shrimp samples were the products of different packers picked up at retail in different cities of the United States. Results of plate counts (average of two plates) varied for individual samples from 24,000 to 60,000,000 per gram. While no enterococci were detected in some samples, counts of this type as high as 55,000 per gram were obtained in others. Coliforms in individual samples varied between none detected and an MPN of 8,850 per gram.

In the past the need for better methods of handling fresh shrimp has been emphasized (2). Several surveys of the bacteriological quality of fresh and frozen shrimp have been made and results reported in the literature (3, 5, 7, 8). Also, a comparison of tests for coliform bacteria and tests for enterococci has been made to determine which type of count is a better index of the sanitary quality of frozen foods (4, 6).

Considering the interest of regulatory agencies in the bacterial quality of foods, which has been especially emphasized in recent years, and since data from tests made some years ago are available, it was considered worthwhile to reexamine frozen raw breaded shrimp bacteriologically and to compare tests for coliforms and enterococci as criteria for the sanitary quality of this product.

METHODS

Samples of breaded frozen shrimp were collected at retail outlets (four samples for each packer's product) and shipped to a laboratory in dry ice. At the laboratory, the package was disguised to prevent packer identification. They were then coded and placed in storage at temperatures below -18 C until again shipped in dry ice to the laboratory at which the

bacteriological tests were to be made. At the testing laboratory, shrimp samples were held at -40 C until they were examined for bacteriological quality.

In examining shrimp samples bacteriologically, the various shrimp within a package were broken up by hitting the intact package with a hard rubber hammer. The package was then opened and a 50 g portion was transferred aseptically to a sterile Waring blender cup. A quantity of 450 ml of sterile, refrigerated dilution water (0.1% peptone) was then added and the sample was blended at high speed for 3 min. Serial dilutions were made from this 1:10 dilution by transferring 10 ml to 90 ml of sterile dilution water, 1 ml of the 1:100 to 9 ml of sterile dilution water, and so on.

Coliforms and *E. coli* tests

In preparing the original sample for tests, 10 ml of the original dilution (1:10) were pipetted into 3 tubes containing Durham tubes in 10 ml of double strength lauryl tryptose broth. Portions of 10 ml of single strength lauryl tryptose broth (3 tubes) were also inoculated with 1 ml of the 1:10 dilution of product (0.1 g of product) and further dilutions were cultured in a similar manner to provide dilutions containing 0.01 and 0.001 g of product. The inoculated medium prepared in this manner was incubated for 48 hr at 35 C and examined for growth and gas formation. Positive tubes were transferred to brilliant green bile lactose broth and incubated for 48 hr. Growth and gas-positive tubes were considered to contain coliform bacteria and were transferred to EC medium and incubated for 24 hr in a water bath at 45.5 ± 0.1 C for 24 hr. The material from gas positive tubes was streaked on eosine methylene blue agar and incubated for 18 to 24 hr at 35 C. EMB agar plates showing typical colonies were considered positive for *Escherichia coli*. Most probable number tables were used to calculate the number of coliforms and *E. coli* per gram of product (1).

Enterococci

One milliliter of the 1:10, 1:100, and 1:1000 dilutions of the original product was plated out (poured plates) on KF streptococcal medium containing 0.01% of triphenyltetrazolium chloride. The KF agar plates were incubated for 48 hr at 35 C after which the number of red colonies were counted and results recorded. Red colonies were spot checked microscopically after staining to determine that they contained chains of 2 or more cocci.

Aerobic plate count

One milliliter of the 1:100, 1:1000, 1:10,000, and 1:100,

¹Contribution No. 1843 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass.

TABLE 1. SUMMARY OF BACTERIAL COUNTS ON RAW BREADED FROZEN SHRIMP

Lot No.	Total plate count (No./g) ¹	Enterococci (No./g) ²	Most probable number of coliforms (No./g) ²	Most probable number of <i>E. coli</i> (No./g) ²
1	630,000	480	<0.3	<0.3
2	490,000	100	10.3	0.7
3	1,900,000	200	3.4	<0.3
4	570,000	2,000	2.2	0.7
5	430,000	2,500	1.7	<0.3
6	720,000	840	1.8	0.3
7	1,500,000	42,000	88.5	6.6
8	3,000,000	4,100	15.3	0.6
9	620,000	120	35.9	<0.3
10	2,400,000	150	7.0	<0.3
11	2,300,000	170	10.0	1.1
12	1,700,000	1,500	6.6	1.2
13	680,000	760	2.6	1.2
14	340,000	2,900	>30.9	2.9
15	1,300,000	7,100	29.3	0.5
16	810,000	<300	5.8	0.4
17	86,000	45	14.0	0.5
18	350,000	190	4.0	<0.3
19	780,000	3,200	12.7	0.1
20	6,100,000	60	61.6	1.4
21	4,900,000	2,000	25.8	1.3
22	1,400,000	7,600	16.8	0.2
23	1,200,000	770	2.2	0.7
24	1,900,000	14,000	58.6	0.8
25	3,500,000	190	6.6	0.6
26	6,200,000	2,200	31.0	<0.3
27	75,000	83	0.3	<0.3
28	15,000	1,400	<0.3	<0.3
29	70,000	<300	12.6	0.1
30	11,000	2,100	<0.3	<0.3
31	5,800,000	1,900	14.5	<0.3
32	1,400,000	17,000	21.8	8.3
33	1,300,000	660	55.8	<0.3
34	930,000	820	24—	0.2

¹Geometric mean of 4 samples

²Arithmetic mean of 4 samples

000 dilutions were plated out (poured plates) on Eugon agar containing 0.5% of yeast extract. Eugon agar plates prepared in this manner were counted and results recorded.

RESULTS AND DISCUSSION

Results of bacteriological tests on raw breaded frozen shrimp are summarized in Table 1. These results include the geometric mean or arithmetic average for four samples in each of 34 lots of the product of different packers.

In these tests made on 136 individual samples of raw breaded frozen shrimp the aerobic plate count on 65 samples (approximately 48%) was higher than 10^6 per gram. The geometric mean of the aerobic plate count on four samples each from 34 lots varied between 86,000 and 6,200,000 per gram. The results of tests for coliform bacteria and for *E. coli* seemed

to have little relationship to the aerobic plate count; but *E. coli* was absent in 1 g portions of approximately 64% of the individual samples and, when present, were low in number compared to the total number of coliforms. When the aerobic plate count for individual samples was $> 10^6$ per gram, the enterococcal count was relatively high in 50 individual samples and relatively low in 14 individual samples.

In tests made on frozen raw breaded shrimp in these laboratories in 1961 (7), 14 out of 56 (28%) individual samples had an aerobic plate count higher than 10^6 per gram. The geometric mean of these counts varied between 100 and 3,500,000 per gram.

In 1967, 564 individual samples of raw breaded frozen shrimp from plants with poor sanitation and 297 samples from other plants were examined bacteriologically by Surkiewicz et al. (8). The geometric mean for aerobic plate counts on product from plants operating under poor sanitary conditions varied between 440,000 and 14,000,000 per gram. The geometric mean of the same type of counts made on product from plants operating under good conditions of sanitation varied between 49,000 and 7,000,000 per gram and was under 10^6 per gram in 81% of the individual samples. This count for product from plants operating under poor sanitary conditions was rarely under 10^6 per g. *Escherichia coli* was absent in 80% of the 0.1 gram portions from plants operating under good conditions of sanitation.

In tests reported here it is not possible to distinguish among products from plants operating under good sanitary conditions and those from plants operating under poor sanitary conditions. However, the aerobic plate count on samples recently examined are generally higher than those obtained in these laboratories in 1961 on the same type of samples. Since the test procedures used in 1961 were different than those reported herein, a valid comparison of the results of the two tests cannot be made. It is considered that plants using good raw material and operating under good conditions of sanitation should be able to produce raw frozen breaded shrimp having a plate count of $< 10^6$ per gram.

Counts for coliforms and for *E. coli* obtained on samples recently examined appeared to have little relationship to the aerobic plate count. In 12 of 17 instances in which the plate count (geometric mean of 4 samples) was greater than 10^6 per gram, the enterococcal count was greater than 500 per gram. On the other hand, in 8 instances in which the enterococcal count (geometric mean of 4 samples) was greater than 500 per gram the plate count was $< 10^6$ per gram.

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INTERIM REPORT OF THE COMMITTEE ON APPLIED LABORATORY METHODS, 1970-1971

During the past 3 years, the International Association of Milk, Food, and Environmental Sanitarians committee on Applied Laboratory Methods participated in the following activities:

(a) Assisted in the preparation of Chapter manuscripts for APHA *Standard Methods for the Examination of Dairy Products*—13th edition (SMEDP). Four of the eight committee members served as Chapter Chairmen for the revision of five Chapters. One ALM member was also a Member of the Intersociety Council. The remaining four served on Chapter subcommittees.

(b) Assisted in development of a continuing program for evaluation of new methods for the examination of dairy products. Collaborative studies were conducted on new and old laboratory methods prior to "Official" identification as "Standard Methods." A mechanism was developed to recognize, by Intersociety Council sanction, methods "Approved" during the interim period between editions of SMEDP.

(c) Developed a program for evaluating food microbiological methods and determining method variances. This program will identify the areas of potential problems in existing and new microbiological methods of examination for different types of foods.

(d) Encouraged development of criteria for certification of media, reagents, materials, and instrumentation in all laboratory concerned with consumer protection.

(e) Dissolved the ALM subcommittee on Laboratory Methods for the Examination of Foods for an unspecified interval. The purpose of this action was to allow formation of an Intersociety Committee on Methods for the Examination of Foods. Although most subcommittee members were reassigned to other subcommittees, additional information relative to participation in the proposed "Intersociety Committee," has not been received.

Subcommittee on Laboratory Methods for the Examination of Water and Other Environmental Samples

Three years ago, this subcommittee was formed to conduct collaborative and independent studies on microbiological methods pertaining to coliforms and other *Enterobacteriaceae* in water and other environmental samples. Little action has resulted. It may be necessary to terminate this subcommittee.

Subcommittee on Laboratory Methods for the Examination of Milk and Milk Products

During the past 3 years, studies by this subcommittee have resulted in more publications on laboratory methods than have the studies of any previous ALM subcommittee during a similar period of time. The subcommittee is to be commended with particular commendation to C. N. Huhtanen, its chairman. In 1970, results of two studies completed by this subcommittee were published in our society publication—*Journal of Milk and Food Technology*. Both publications, "Effect of Dilution Bottle Mixing Methods on the Recovery of Raw Milk Bacteria" and "A Comparison of Horizontal Versus Vertical Mixing Procedures and Plastic Versus Glass Petri Dishes for Enumerating Raw Milk Bacteria," provided SMEDP Chapter Chairmen with the necessary information to prepare the 13th edition of *Standard Methods*. Two additional studies have been completed and will be submitted to the *Journal of Milk and Food Technology* for publication.

Applied Laboratory Methods Committee

A. Richard Brazis, *Chairman*; C. N. Huhtanen, *Subcommittee Chairman*; Martin Favero, *Subcommittee Chairman*; William Arledge; Earl Cook; C. B. Donnelly; Sherman Ferrell; Roy Ginn; J. J. Jezeski; James Messer; Joseph Murphy, Jr.; F. E. Nelson; H. E. Randolph; Edmond Sing; Donald Thompson; Donald Pusch; John C. Hoff; F. F. Busta; R. L. Morris; and Arnold Salinger.

HOLDERS OF 3-A SYMBOL COUNCIL AUTHORIZATIONS ON FEBRUARY 20, 1972

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y-Treas., Dept. of Food Technology, Dairy Industry Bldg., Iowa State University, Ames, Iowa 50010."

0102 Storage Tanks for Milk and Milk Products As Amended

116	Jacob Brenner Company, Inc.	(10/ 8/59)
	450 Arlington, Fond du Lac, Wisconsin 54935	
28	Cherry-Burrell Corporation	(10/ 3/56)
	575 E. Mill St., Little Falls, N. Y. 13365	
102	Chester-Jensen Company, Inc.	(6/ 6/58)
	5th & Tilgham Streets, Chester, Pennsylvania 19013	
1	Chicago Stainless Equipment Corp.	(5/ 1/56)
	5001 No. Elston Avenue, Chicago, Illinois 60630	
2	CP Division, St. Regis	(5/ 1/56)
	100 C. P. Ave., Lake Mills, Wisconsin 53551	
117	Dairy Craft, Inc.	(10/28/59)
	St. Cloud Industrial Park St. Cloud, Minn. 56301	
76	Damrow Company	(10/31/57)
	196 Western Avenue, Fond du Lac, Wisconsin 54935	
115	DeLaval Company, Ltd.	(9/28/59)
	113 Park Street, So., Peterborough, Ont., Canada	
207	The DeLaval Separator Co.	(7/23/69)
	Duchess Turnpike, Poughkeepsie, N. Y. 12602	
109	Girton Manufacturing Company	(9/30/58)
	Millville, Pennsylvania 17846	
114	C. E. Howard Corporation	(9/21/59)
	9001 Rayo Avenue, South Gate, California 90280	
127	Paul Mueller Company	(6/29/60)
	P. O. Box 828, Springfield, Missouri 65801	
197	Paul Mueller (Canada), Ltd.	(9/ 9/67)
	84 Wellington St., South, St. Marys, Ont., Canada	
213	Sanitary Processing Equipment Corp.	(2/18/72)
	Butternut Drive, E. Syracuse, N. Y. 13057	
21	Technova, Inc. Gosselin Division	(9/20/56)
	1450 Hebert c. p. 758 Drummondville, Quebec, Canada	
31	Walker Stainless Equipment Co.	(10/ 4/56)
	Elroy, Wisconsin 53929	

0204 Pumps for Milk and Milk Products Revised, as Amended

214R	Ben H. Anderson Manufacturers	(5/20/70)
	Morrisonville, Wis. 53571	
212R	Babson Bros. Co.	(2/20/70)
	2100 S. York Rd., Oak Brook, Ill. 60621	
29R	Cherry-Burrell Corporation	(10/ 3/56)
	2400 Sixth St., S. W., Cedar Rapids, Iowa 52406	
63R	CP Division, St. Regis	(4/29/57)
	100 C. P. Ave., Lake Mills, Wisconsin 53551	
205R	Dairy Equipment Co.	(5/22/69)
	1919 So. Stoughton Road, Madison, Wis. 53716	
180R	The DeLaval Separator Co.	(5/ 5/66)
	Duchess Turnpike, Poughkeepsie, N. Y. 12602	
65R	G & H Products Corporation	(5/22/57)
	5718 52nd Street, Kenosha, Wisconsin 53140	
145R	ITT Jabco, Incorporated	(11/20/63)
	1485 Dale Way, Costa Mesa, Calif. 92626	

26R	Ladish Co., Tri-Clover Division	(9/29/56)
	9201 Wilmot Road, Kenosha, Wisconsin 53140	
148R	Robbins & Myers, Inc.	(4/22/64)
	Moyno Pump Division 1345 Lagonda Ave., Springfield, Ohio 45501	
163R	Sta-Rite Products, Inc.	(5/ 5/65)
	P. O. Box 622, Delavan, Wisconsin 53115	
72R	L. C. Thomsen & Sons, Inc.	(8/15/57)
	1303 53rd Street, Kenosha, Wisconsin 53140	
219	Tri-Canada Limited	(2/15/71)
	6500 Northwest Drive, Mississauga, Ont., Canada	
175R	Universal Milking Machine Div.	(10/26/65)
	National Cooperatives, Inc. First Avenue at College, Albert Lea, Minn. 56007	
52R	Viking Pump Div.	
	Houdaille Industries, Inc.	(12/31/56)
	406 State Street, Cedar Falls, Iowa 50613	
5R	Waukesha Foundry Company	(7/ 6/56)
	Waukesha, Wisconsin 53186	

0402 Homogenizers and High Pressure Pumps of the Plunger Type, As Amended

87	Cherry-Burrell Corporation	(12/20/57)
	2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404	
37	CP Division, St. Regis	(10/19/56)
	Fort Atkinson, Wis. 53538	
75	Gaulin, Inc.	(9 /26/57)
	44 Garden Street, Everett, Massachusetts 02149	

0506 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service, As Amended

131R	Almont Welding Works, Inc.	(9/ 3/60)
	4091 Van Dyke Road, Almont, Michigan 48003	
98R	Beseler Steel Products, Inc.	(3/24/58)
	417 East 29th, Marshfield, Wisconsin 54449	
70R	Jacob Brenner Company	(8/ 5/57)
	450 Arlington, Fond du Lac, Wisconsin 54935	
40	Butler Manufacturing Co.	(10/20/56)
	900 Sixth Ave., S. E., Minneapolis, Minn. 55114	
118	Dairy Craft, Inc.	(10/28/59)
	St. Cloud Industrial Park St. Cloud, Minn. 56301	
66	Dairy Equipment Company	(5/29/57)
	1818 So. Stoughton Road, Madison, Wisconsin 53716	
123	DeLaval Company, Ltd.	(12/31/59)
	113 Park Street, South Peterborough, Ont., Canada	
45	The Heil Company	(10/26/56)
	3000 W. Montana Street, Milwaukee, Wisconsin 53235	
201	Paul Krohnert Mfg., Ltd.	(4/ 1/68)
	West Hill, Ontario, Canada	
80	Paul Mueller (Canada), Ltd.	(11/24/57)
	84 Wellington Street, So., St. Marys, Ont., Canada	
85	Polar Manufacturing Company	(12/20/57)
	Holdingsford, Minn. 56340	
144	Portersville Equipment Company	(5/16/63)
	Portersville, Pennsylvania 16051	
71	Progress Industries, Inc.	(8/ 8/57)
	400 E. Progress Street, Arthur, Illinois 61911	
121	Technova Inc. Gosselin Division	(12/ 9/59)
	1450 Hebert c. p. 758 Drummondville, Quebec, Canada	

- 47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Ave., North Kansas City, Mo. 64116
- 189 A. & L. Tougas, Ltée (10/ 3/66)
1 Tougas St., Iberville, Quebec, Canada
- 25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

**0808 Fittings Used on Milk and Milk Products
Equipment, and Used on Sanitary Lines Conducting
Milk and Milk Products, Revised**

- 79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186
- 138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)
103 Rivalda Rd., Weston, Ont., Canada
- 82R Cherry-Burrell Corporation (12/11/57)
2400 Sixth Street, S.W. Cedar Rapids, Iowa 52406
- 124R DeLaval Company, Ltd. (2/18/60)
113 Park Street, South Peterborough, Ont., Canada
- 184R The DeLaval Separator Co. (8/ 9/66)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 67R G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140
- 199R Grayco, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413
- 203R Grinnell Company (11/27/68)
260 W. Exchange St., Providence, R. I. 02901
- 218 Highland Equipment Corporation (2/12/71)
74-10 88th St., Glendale, N.Y. 11227
- 204R Hills McCanna Company (2/10/69)
400 Maple Ave., Carpentersville, Ill. 60110
- 34R Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140
- 206R Paul Mueller Co. (3/ 5/68)
P. O. Box 828, Springfield, Mo. 65601
- 149R Q Controls (5/18/64)
Occidental, California 95465
- 227 Stainless Steel Craft Corporation (1/11/72)
4503 Alger Street, Los Angeles, California
- 89R Sta-Rite Industries, Inc. (12/23/68)
P. O. Box 622, Delavan, Wis. 53115
- 73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140
- 191R Tri-Canada Fittings & Equipment, Ltd. (11/23/66)
21 Newbridge Road, Toronto 18, Ontario, Canada
- 151R Tubular Components, Inc. (11/18/64)
Butternut Drive, East Syracuse, New York 13057
- 215R Universal Milking Machine Div., (7/31/70)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007
- 86R Waukesha Specialty Company, Inc. (12/20/57)
Walworth, Wisconsin 53184

**0902 Thermometer Fittings and Connections Used
on Milk and Milk Products Equipment and
Supplement 1, As Amended**

- 32 Taylor Instrument Process Control,
Div. Sybron Corp. (10/ 4/56)
95 Ames Street, Rochester, New York 14611
- 206 The Foxboro Company (8/11/69)
Neponset Ave., Foxboro, Mass. 02035

**1002 Milk and Milk Products Filters Using Disposable
Filter Media, As Amended**

- 35 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th Street, Kenosha, Wisconsin 53140

**1102 Plate-Type Heat Exchangers for Milk and Milk
Products, As Amended**

- 20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207
- 30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404
- 14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013
- 38 CP Division, St. Regis (10/19/56)
Fort Atkinson, Wisconsin 53538
- 120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South Peterborough, Ont., Canada
- 17 The DeLaval Separator Company (8/30/56)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

**1202 Internal Return Tubular Heat Exchangers,
for Milk and Milk Products, As Amended**

- 103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013
- 96 C. E. Rogers Company (3/31/64)
8731 Witt Street, Detroit, Michigan 48209
- 152 The DeLaval Separator Co. (11/18/69)
350 Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 217 Girton Manufacturing Co. (1/23/71)
Millville, Pa. 17846
- 225 Sanitary Processing Equipment Corporation (11/24/71)
Butternut Drive, East Syracuse, N.Y.

**1303 Farm Milk Cooling and Holding Tanks—
Revised, As Amended**

- 11R CP Division, St. Regis (7/25/56)
100 C. P. Ave.
Lake Mills, Wisconsin 53551
- 4R Dairy Equipment Company (6/15/56)
1919 S. Stoughton Road, Madison, Wisconsin 53716
- 92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South Peterborough, Ontario, Canada
- 49R The DeLaval Separator Company (12/ 5/56)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846
- 95R Globe Fabricators, Inc. (3/14/58)
7744 Madison Street, Paramount, California 90723
- 179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road, Preston, Ont., Canada
- 12R Paul Mueller Company (7/31/56)
P. O. Box 828, Springfield, Missouri 65801
- 58R Schweitzer's Metal Fabricators, Inc. (2/25/57)
806 No. Todd Avenue, Azusa, California 91702
- 134R Universal Milking Machine Division (5/19/61)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007
- 216R Valco Manufacturing Company (10/22/70)
3470 Randolph St., Huntington Pk., Calif. 90256
- 42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126
- 18R Whirlpool Corporation, St. Paul Division (9/20/56)
850 Arcade Street, St. Paul, Minnesota 55106
- 55R Superior Metalware Industries,
(Formerly John Wood Company) (1/23/57)
509 Front Avenue, St. Paul, Minnesota 55117
- 170R The W. C. Wood Co., Ltd. (8/ 9/65)
5 Arthur Street, South, Box 750, Guelph, Ont., Canada

- 16R Zero Manufacturing Company (8/27/56) 160 Dairy Craft, Inc. (4/ 5/65)
Washington, Missouri 63090
St. Cloud Industrial Park
St. Cloud, Minn. 56301

**1400 Inlet and Outlet Leak Protector Plug Valves
for Batch Pasteurizers, As Amended**

- 122R Cherry-Burrell Corporation (12/11/59)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406
69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140
27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140
78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140
- 181 Damrow Company (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935
156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue, South Gate, California 90280
155 Paul Mueller Co. (2/10/65)
P. O. Box 828, Springfield, Missouri 65801
195 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada
165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

**1603 Evaporators and Vacuum Pans for Milk and
Milk Products, Revised**

- 132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207
11R Blaw-Knox Food & Chemical Equip., Inc. (2/12/59)
P. O. Box 1041
Buffalo, N. Y. 14240
110R Arthur Harris & Company (11/10/58)
210-218 North Aberdeen Street, Chicago, Illinois 60607
164R Mora Industries, Inc. (4/25/65)
999 E. Maple Street, Mora, Minnesota 55051
107R C. E. Rogers Company (8/ 1/58)
8731 Witt Street, Detroit, Michigan 48209
186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48010
- 174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109
209 Dobby Packaging Machinery (7/23/69)
Domain Industries, Inc.
869 S. Knowles Ave., New Richmond, Wis. 54017
222 Maryland Cup Corporation (11/15/71)
Owings Mills, Maryland 21117
193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

2400 Non-Coil Type Batch Pasteurizers

**1702 Fillers and Sealers of Single Service Containers,
For Milk and Milk Products, As Amended**

- 221 Bertopack Limited (5/25/71)
75 Ardel Place, Kitchener, Ontario, Canada
192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404
139 Exact Weight Scale Company (4/15/68)
944 W. 5th Ave., Columbus, O. 43212
137 Ex-Cell-O Corporation (10/17/62)
P. O. Box 386, Detroit, Michigan 48232
220 Haskon, Inc., Package Equipment Division (4/27/71)
2285 University Ave., St. Paul, Minnesota 55114
142 Polygal Company (4/15/63)
Div. of Inland Container Corp.
P. O. Box 68074, Indianapolis, Indiana 46268
211 Twinpak, Inc. (2/ 4/70)
2225 Hymus Blvd., Dorval 740, P.Q.
- 161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365
158 CP Division, St. Regis (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551
187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
208 The DeLaval Separator Co., (7/23/69)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

**2500 Non-Coil Type Batch Processors for Milk and
Milk Products**

**1901 Batch and Continuous Freezers, For Ice Cream,
Ices and Similarly Frozen Dairy Foods, As Amended**

- 141 CP Division, St. Regis (4/15/63)
100 C. P. Avenue, Lake Mills, Wisconsin 53551
146 Cherry-Burrell Corporation (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404
- 162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N. Y. 13365
159 CP Division, St. Regis (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551
188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
196 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada
202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950
- 2600 Sifters for Dry Milk and Dry Milk Products**
- 171 Entoleter, Inc. (9/ 1/65)
Subsidiary of American Mfg. Co.
251 Welton St., Hamden, Connecticut 06514
173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P. O. Box 1041
Buffalo, New York 14240
185 The Orville-Simpson Co. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223

176	Sprout, Waldron & Co., Inc. Munsey, Pennsylvania 17756	(1/ 4/66)	226	Fischer & Porter Company County Line Road, Warminster, Pa. 18974	(12/ 9/71)
172	SWECCO, Inc. 6111 E. Bandini Blvd., Los Angeles, California 90022	(9/ 1/65)	224	The Foxboro Company Foxboro, Massachusetts 02035	(11/16/71)

**2800 Flow Meters for Milk and
Liquid Milk Products**

223	C-E IN-VAL-CO, a division of Combustion Engineering, Inc. P. O. Box 556, 3102 Charles Page Blvd., Tulsa, Oklahoma 74101	(11/15/71)
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**QUALITY EVALUATION OF YOGURT PRODUCED
COMMERCIALY IN ONTARIO**

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(Received for publication November 4, 1971)

ABSTRACT

Analyses for fat, total solids, solids-not-fat (SNF), pH, and net weight were conducted on 152 yogurts of which 15 were unflavored. These yogurts were produced by 13 different manufacturers and sold in Ontario. The fat content of all samples varied from 0.9 to 3.6% with a mean of 1.98%. For the plain yogurt, the mean fat percentage was 2.46%. The SNF content of all samples ranged from 10.0 to 28.9% with a mean of 18.96%. For the plain yogurt, the mean SNF percentage was 13.40. The pH values for all samples varied from 3.27 to 4.53. The mean overweight for all samples was 7.2% with as much as 15% for one manufacturer.

The success of yogurt as a dairy product on the Canadian market has resulted in the introduction of many new brand names. Most dairies producing these brands have had little or no previous experience in yogurt manufacture. Lack of regulatory standards of composition has allowed the dairies to experiment with fat and solids-not-fat (SNF) content of yogurt. This has resulted in general confusion as to the composition of yogurts available to the consumer in Canada. We have undertaken the study reported here to make available to the dairy industry and the regulatory agencies information regarding the composition of yogurt marketed in Ontario.

MATERIALS AND METHODS

Products

One hundred and fifty-two commercially produced yogurts were obtained from grocery stores in Ontario during June, July, and August of 1971. The samples which represented 13 different manufacturers included 15 plain and 137 fruit or fruit-flavored yogurts (Swiss style). All products were trans-

ported to the laboratory within 2 hr of purchase and kept refrigerated until analyzed.

Analyses

Before removing portions of the sample for analysis, the whole content of the container was transferred quantitatively into a Waring blender and thoroughly mixed for 3 min at high speed. Analyses for fat and total solids were done in duplicate.

Fat, total solids, solids-not-fat

Percent fat and total solids were determined by the Mojonnier method (1). Solids-not-fat was considered to be the arithmetic difference.

Weight of product

The outside of each container of yogurt was wiped dry and the container and contents weighed. After removal of the product, each container was washed, dried, and reweighed. The difference in weight represented the net weight of product and was reported with the weight specified on the package.

pH

A Radiometer 26 pH meter with a combination electrode was used to determine the pH of each sample.

RESULTS

Samples were analysed and grouped according to brand. Each of the groups A to H inclusive, represented one brand and contained 15 to 17 samples. Group I represented five different brands of yogurt and contained 20 samples. Results are summarized in Table I.

Fat

The fat content ranged from 0.9 to 3.6%. With-

TABLE I. ANALYSES OF COMMERCIAL SAMPLES OF YOGURT

Group	A	B	C	D	E	F	G	H	I	Total
Samples tested	15	16	16	17	17	17	17	17	20	152
Plain samples	0	0	0	2	1	0	3	2	7	15
Fat %										
Mean	2.90	1.04	1.10	2.04	3.09	1.63	2.07	1.63	2.27	1.98
Range ¹	2.5-3.2	0.9-1.2	0.9-1.5	1.3-3.0	2.5-3.6	1.2-2.1	1.5-2.7	1.1-3.4	1.4-3.2	0.9-3.6
Range ²	2.5-3.2	0.9-1.2	0.9-1.5	1.3-3.0	2.5-3.6	1.2-2.1	1.5-2.5	1.1-2.8	1.4-2.9	0.9-3.6
SNF %										
Mean	21.98	23.43	23.33	21.28	24.47	21.87	20.87	23.41	17.40	21.79
Range ¹	19.8-24.4	18.7-28.5	21.2-25.7	12.6-25.0	18.9-26.6	21.1-22.6	17.1-24.4	13.4-28.9	10.0-24.3	10.0-28.9
Range ²	19.8-24.4	18.7-28.5	21.2-25.7	18.9-25.0	19.2-26.6	21.1-22.6	19.8-24.4	21.8-28.9	17.6-24.3	17.6-28.9
pH										
Mean	3.66	3.92	3.89	4.09	3.87	4.01	3.93	3.82	3.97	3.91
Range ¹	3.27-3.97	3.77-4.06	3.77-4.08	3.85-4.25	3.71-4.15	3.78-4.28	3.60-4.40	3.68-3.95	3.53-4.53	3.27-4.53
Range ²	3.27-3.97	3.77-4.06	3.77-4.08	3.85-4.25	3.71-4.15	3.78-4.28	3.60-4.40	3.68-3.95	3.53-4.53	3.27-4.53
Weight										
Mean % over or under fill	+10	+3	+11	+7	+11	+11	+15	-1	+4	+7.2

¹Range for all samples²Range for all but plain samples

in groups the range interval was narrower but was still greater than 1% for 5 of the 9 groups. The mean of all samples was 1.98% fat and for the plain yogurt, 2.46%. When the latter was excluded from the groups, some of the ranges were narrowed. The means of the groups varied from 1.04 to 3.09%. Only three of the manufacturers, groups B, C, and F, had the fat content (1%) advertised on the packages. In these instances the actual percent fat ranged from 0.9 to 1.2, 0.9 to 1.5, and 1.2 to 2.1 with means of 1.04, 1.10, and 1.63, respectively.

SNF

The SNF content of all samples ranged from 10.0 to 28.9%. When the plain yogurt was excluded, the range was narrowed to 17.6 to 28.9%. The range interval within groups was as narrow as 1.5% for group F and as large as 15.5% for group H. The range interval became less than 10% for each group when plain samples were omitted. The mean of all samples was 21.79% and for plain yogurt, 13.04%.

pH

The pH values of all samples varied from 3.27 to 4.53 with a mean value of 3.91. The smallest range was 0.27 for group H and the largest was 1.0 for group I. There did not appear to be any correlation between pH and the type of fruit added to yogurt.

Weight

The packages contained a mean overweight of 7.2%. The group means varied from plus 15% in G to minus 1% in H.

DISCUSSION

The results clearly show that yogurt sold on the Ontario market varies considerably in fat and SNF content. Differences between manufacturers could be expected but the wide variations in composition within yogurts of a single manufacturer were surprising. Plain yogurt was generally higher in fat, probably because it was not being diluted by the addition of fruit or flavorings. It is also obvious that addition of high-solids fruit preparations will raise the SNF content above that of plain yogurt. Depending upon the type of fruit, more or less of it must be added to yogurt to give it the desired flavor. This will result in varying dilution effects with respect to fat content. This explains, in part, the variations in fat content between flavors. However, it does not explain wide variations within a flavor of a single manufacturer such as 1.2 to 2.8% fat in strawberry of group H, or 1.2 to 2.1% fat in orange of group F, or 1.8 to 3.0% fat in banana of group D. That fat content can be controlled more closely was demonstrated by the results for group B in which the fat range interval for 16 samples of 7 different flavors was only 0.3%.

The same remarks are generally true for the SNF content. Even when plain yogurt samples were omitted, SNF range intervals were as great as 9.7 for group B. However, group F with seven flavors represented in 17 samples had a range interval of only 1.5%.

It is difficult to draw any conclusions from the

data on pH with regard to adequate pH control by the manufacturer. Since the samples were collected from retail outlets, and since yogurt is a biologically active product and therefore has to be kept at about 5 C, it is likely that inadequate storage conditions and age of sample influenced the pH readings obtained. In this respect, the main factor may be the type of bacterium which predominates in the product. An excess of *Lactobacillus bulgaricus* would cause a lower pH than would an excess of *Streptococcus thermophilus*.

Generally the customer received good measure for his money. On the average, the weights were in excess by 7.2%. Samples in groups I and B were well controlled with excess mean weights of only 4 and 3%, respectively. Group B was exceptionally well controlled with only 1 sample of 16 being un-

derweight and that by less than 1 g.

Based on the results of this study, yogurt of uniform composition is generally not available to the consumer. This was evident not only between brands but within most brands. This lack of consistency in product composition may also be responsible for variations in viscosity of the product and thus influence public acceptance.

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ECOLOGY OF THE LACTIC STREPTOCOCCI. A REVIEW¹

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ABSTRACT

Early and recent literature on the nomenclature and sources of lactic streptococci in nature is reviewed. Evidence is presented in favor of including *Streptococcus diacetylactis* as a recognized species in the group N streptococci. Despite many research efforts, the natural habitat of *Streptococcus cremoris* remains unknown. An appeal for more ecological studies on these important bacteria is made.

During the past several years, the need to isolate new lactic streptococcus starter culture strains from raw milk and other natural sources has been emphasized by cheesemakers, industry consultants, and research workers. Several factors have contributed to this need for strains of lactic streptococci previously unused in dairy fermentations. Among these have been frequent starter culture failures resulting from virus infection of existing strains, undesirable flavors encountered in cultured dairy products, and insufficient development of acid during fermentation.

At the present time there are no known cultures in use in the dairy industry which are resistant to lysis by bacteriophages. Some strains of lactic streptococci are susceptible to only one race of phage, whereas others are lysed by several known races (29). Repeated use of the same cultures has increased the phage problem and therefore newly isolated cultures resistant to existing bacteriophages are in great demand.

Over the past 10 years the dairy industry has experienced increased incidence of a fruity flavor defect in Cheddar cheese. Research (4, 69) has indicated there may be a relationship between ethanol, fatty acid, and total carbonyl production by the lactic streptococci and the fruity flavor defect. Also, cultures of *Streptococcus cremoris* have been found (69, 70) to be the most suitable for producing aged grade AA Cheddar cheese. Since this flavor defect results in a large economic loss to the dairy industry each year, isolation and selection of starter cultures, especially *S. cremoris*, is desirable.

It is well known that the fast acid-producing ability of a lactic streptococcus starter culture is not a stable property. For example, a single strain of a fast acid-producing culture has been shown to contain at least 2% slow acid-producing cells (12, 26). For unknown reasons, these slow cells sometimes overtake the fast cells and a sudden occurrence of a slow acid-producing culture results. In the manufacture of dairy products such as Cheddar cheese this can have disastrous economic consequences, and it has become necessary to isolate fast cultures from different sources and make them available on a continuing basis to the dairy fermentation industry.

DEFINITION OF LACTIC STREPTOCOCCI

While *Bergey's Manual of Determinative Bacteriology* (6) only recognizes 2 species of lactic streptococci, it is clear that *Streptococcus diacetylactis* should also be included (8, 57, 58, 68). The principal distinguishing characteristics of the three species are shown in the following table (58, 60, 62, 63, 65, 74).

	Growth							Serological group	
	10 C	40 C	45 C	pH 9.2	4.0% NaCl	NH ₃ from arginine ¹	King's test ²		CO ₂ from citrate ³
<i>S. lactis</i>	+	+	-	+	+	+	-	-	N
<i>S. cremoris</i>	+	-	-	-	-	-	-	-	N
<i>S. diacetylactis</i>	+	+	-	+	+	+	+	+	N

¹See Niven, et al. (48); an occasional isolate of *S. diacetylactis* may be negative (58).

²A test for diacetyl plus acetoin (34); diacetyl-negative mutants are known (9).

³Incubated 24 hr at 30 C in citrate broth (58).

Differential agar (51, 55) and broth (54) media for these three species have been described.

DNA BASE COMPOSITION

Deoxyribonucleic acid (DNA) base composition of the lactic streptococci has been studied by at least two groups of workers. Knittel (36) examined the DNA of lactic streptococci in comparison to that isolated from representatives of the other streptococci.

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ccal groups. His data showing the average moles percent of guanine plus cytosine (G + C) may be summarized as follows:

Source of DNA	% G + C
<i>S. lactis</i> ^a	36.1 ^b
<i>S. diacetylactis</i> ^a	35.1 ^c
<i>S. cremoris</i> ^a	36.8 ^d
<i>S. pyogenes</i> ^e	38.0
<i>S. agalactiae</i> ^e	35.5
<i>S. salivarius</i> ^f	41.2
<i>S. bovis</i> ^f	39.0
<i>S. thermophilus</i> ^f	40.0
<i>S. uberis</i> ^f	36.8
<i>S. faecalis</i> ^g	37.1
<i>S. durans</i> ^g	37.6

^aMember of the lactic group.

^bAverage of 6 strains with range from 33.8 to 36.9.

^cAverage of 5 strains with range from 33.6 to 34.8.

^dAverage of 5 strains with range from 35.0 to 36.2.

^eMember of the pyogenic group.

^fMember of the viridans group.

^gMember of the enterococcus group.

The similarity in base compositions, especially within the lactic group, is apparent but more complete data would be required to make conclusions about genetic relatedness of the streptococci. In this regard, Miller and Morgan (46) carried out DNA-RNA hybridization experiments to test genetic homology of lactic streptococci. All strains examined had similar base compositions; no hybridization occurred between *Streptococcus lactis* or *Streptococcus multigenes* and *Streptococcus faecalis* DNA. Hopefully a complete study of the relationship between all the streptococci using DNA-DNA hybridization will be made in the near future.

EARLY HISTORY

Use of starter cultures of lactic acid bacteria to induce the souring and enhance the flavor of milk and cream has been practiced for centuries. In the late nineteenth century, scientists began to investigate the changes caused by starters and three independent discoveries revealed the cause of milk souring reactions. Storch (67) in Denmark, Conn (13) in the United States, and Weigmann (72) in Germany concluded that the souring of milk or ripening of cream was dependent upon the bacteria present. These workers instituted programs of preparing fresh starter for daily use in ripening cream to be used for buttermaking. Furthermore in 1890, Storch applied for the first U. S. patent on a method of producing a starter culture which later was granted (U. S. patent No. 561,291, June 2, 1896).

Conn (14) indicated that cream ripening involved more than just souring since addition of acid did not accomplish the same results as bacterial growth. He pointed out that acid and flavor should probably be

regarded as separate fermentations, with the acid coming from milk sugar (lactose) and the flavor constituents arising from some other source. He also stated that aroma should be separated from flavor and that it is more unusual for an organism to produce aroma than flavor.

Storch (67) investigated milk-souring bacteria isolated from different sources and found that different acid-producing forms induced very different aromas and flavors.

The first suggestion of combining acid and aroma-producing bacteria for use in ripening cream came from Weigmann (72). He obtained fine-flavored butter with good keeping quality by manufacturing it from cream ripened with acid and aroma-producing bacteria.

In 1919, Orla-Jensen (49) published a monograph which presented results of an intensive investigation of the lactic acid bacteria. A detailed morphological and biochemical description of the two organisms responsible for acid production in milk and cream was given. The first was designated *S. lactis* and other names by which this organism had been known in the literature were mentioned by him. These were *Bacterium lactis*, *Streptococcus acidi lactici*, *Bacterium lactis acidi*, *Bacterium guntherii*, and *Streptococcus lacticus*. This organism was first called *Bacterium lactis* when isolated by Lister (41) in 1878, from soured milk using the dilution method. Orla-Jensen described *S. lactis* as the predominating diplococcus of sour milk, often somewhat longer than broad. Its optimum temperature was 30 C and it either lacked or had weak powers of fermentation toward sucrose, raffinose, inulin, or starch. It used levulose, glucose, mannose, galactose, and generally mannitol. Some strains fermented neither arabinose or xylose while others used both. At least one strain studied very soon lost the power of forming acid in milk.

The second milk-souring bacterium described by Orla-Jensen was given the name *S. cremoris* (from cream). The author stated that this was the lactic acid bacterium first studied by Storch (67) in 1890 which was capable of aroma formation. It was mentioned that *Bacterium lactis longi* and *Streptococcus hollandicus* were slime-forming varieties of this species. The optimum temperature was given as below 30 C and the organism had the ability to form carbonic acid so that fine stripes appeared in the curd. It attacked lactose, levulose, glucose, and mannose, but rarely sucrose, maltose, dextrin, alcohols, and pentoses. *Streptococcus cremoris* generally formed long chains both in milk and broth.

This author (49) also devoted considerable effort to a study of a group of bacteria which he placed in

the genus *Betacoccus* (from beets). They were described as bacteria associated with green plant matter and juicy fruits. They entered milk supplies by being introduced with plant feed into the intestinal tract of animals and then into the milk. Two species were initially recognized, *Betacoccus arabinosaceus* and *Betacoccus bovis*. The first species fermented arabinose and often xylose, the second species only xylose or no pentoses. This genus was separated from the *Streptococcus* genus on the following basis: streptococci formed dextro-rotatory lactic acid and only traces of by-products, while betacocci formed a number of by-products in addition to levorotatory lactic acid.

In 1919, announcements by Hammer and Bailey (24) and Boekhout and Ott de Vries (5) provided information on the possible identity of additional species of bacteria important in milk fermentations. These workers independently demonstrated that pure cultures of *S. lactis* would not produce a volatile acidity at all comparable with that produced by a good starter. Furthermore, starters generally contained an associated organism which, when grown in combination with *S. lactis*, developed volatile acidity essentially the same as that produced by a satisfactory starter. Hereafter, these flavor bacteria generally became known as associative organisms. Hammer (23) made a thorough study of these associative organisms. He recognized at least 2 types of them which he generally designated as A and B and later specifically as *Streptococcus citrovorus* and *Streptococcus paracitrovorus*, respectively. He experimentally demonstrated the following with regard to *S. citrovorus*: it could easily be isolated by direct plating and also by repeated transfer on whey agar slopes; it was isolated repeatedly from starters being carried in creameries; it combined non-specifically with different *S. lactis* organisms to produce a high volatile acidity, especially when inoculated 1 to 4 days before *S. lactis*; when it was destroyed by heat after a considerable period of growth and *S. lactis* was inoculated, a low volatile acidity essentially like that produced by *S. citrovorus* alone resulted; when it was added to milk in which *S. lactis* had produced a small amount of acid and then had been destroyed by heat, a high volatile acidity was obtained; it produced a higher volatile acidity in milk to which a small amount of sterile lactic acid had been added than in milk without the acid; and it produced volatile acid from sterile citric acid added to milk, which suggested that citric acid normally present in milk was the source of volatile acid developed when *S. citrovorus* was grown in pure culture in milk. For *S. paracitrovorus* he showed the following: the volatile acidity produced by this bacterium when grown

alone was higher than that produced by *S. citrovorus* and usually was as high as that produced by a good starter culture; it could be isolated by direct plating; it occurred less frequently in starters than *S. citrovorus*; addition of sterile citric acid to milk increased its volatile acid production; and it produced some lactic acid in milk, some of which later seemed to be changed to volatile acid.

Additional proof that starters contained more than one species of bacteria was provided when Hammer (22) compared the type of lactic acid produced in a starter and in pure cultures of *S. lactis*. He found that starters contained some inactive lactic acid and therefore could not be considered pure cultures of *S. lactis* since this organism produced only the dextro-rotatory form of the acid.

At about this same time (1926) Orla-Jensen et al. (50) presented a paper in which they indicated that the aroma bacteria of milk belonged to the genus, *Betacoccus*. Their report is worthy of some detailed consideration. The authors pointed out that these bacteria formed small amounts of acetic and carbonic acids and other volatile products but hardly any appreciable quantities of lactic acid. They manufactured butter from cream ripened with starters consisting of lactic acid and aroma bacteria and failed to obtain consistent aroma production. From this they concluded that butter of good aroma could only be produced by use of vigorous pure cultures of Storch's cream-ripening bacterium which had been studied and named *S. cremoris* by Orla-Jensen earlier (49). They considered the possibility that the aroma bacteria might be non-acid-producing forms of *S. cremoris* but found ample reason to discard this assumption upon confirming the previous report of Hammer (23) that they fermented citric acid. Freshly isolated cultures were able to cause vigorous evolution of carbon dioxide in milk when citrates were added, but they completely lost this power after propagation in milk for some time as pure cultures. Since this property was not a constant one, the authors felt it was of little taxonomic significance. But since *B. arabinosaceus* and *B. bovis* strains also fermented citrate and had low optimum growth temperatures (20 C), they felt the aroma bacteria were related to this genus. Since the betacocci were distinguished from streptococci primarily by formation of levorotatory lactic acid, these investigators endeavored to show that the aroma bacteria were betacocci by demonstrating their production of this type of acid. Since milk did not seem to be a favorable medium for the aroma bacteria in pure cultures, they attempted to fortify it with different substances. It was found that with 10 to 50% yeast extract in milk, these organisms formed just as much acid as other strepto-

cocci so they were able to analyze the acid produced. It was found to be mostly lactic acid of the levo-rotatory type. These workers therefore concluded that the aroma bacteria were members of the *Betacoccus* genus and not degenerate forms of *S. cremoris*. Von Freudenreich et al. (17) had also worked with slow milk-souring bacteria and the name *Streptococcus kefir* was used for these organisms because von Freudenreich had isolated them originally from kefir. It was generally agreed that these were the same bacteria studied by Orla-Jensen (49).

In 1929, Knudsen and Sorensen (38) proposed the name *Betacoccus cremoris* for the aroma bacteria. They distinguished two types, a and b, the former being quite similar to the *S. paracitrovorus* of Hammer (23). Knudsen (37) subsequently wrote a review article on starters, citing much of the early work done through 1930.

At this point it would be well to mention something about *Leuconostoc mesenteroides*. This organism is not used in controlled dairy fermentations because it produces undesirable end products, but nevertheless, it is historically related to the aroma bacteria. Galesloot (18) summarized three early reports on this organism by Cienkowski, Van Tiegham, and Migula dated from 1878 to 1900. They described it as a slime-producing streptococcus found in slimy sugar solutions and fermenting vegetable and plant materials. Originally called *Ascococcus mesenteroides*, the genus name was changed to *Leuconostoc* by Van Tiegham because the organisms were morphologically similar to blue-green algae of the *Nostoc* genus. This was in the year 1878, and prior to the time (1919) the genus name of *Betacoccus* was proposed by Orla-Jensen (49).

In 1928, Hucker and Pederson (32) investigated a large number of low acid-producing streptococci isolated from slimy sugar solutions, fermenting vegetables, and milk and milk products. They felt that these types of streptococci from such sources were identical or at least closely related and therefore belonged in a single genus. All strains studied produced about 45% levo-rotatory lactic acid from glucose, 20% CO₂ and 25% volatile products including acetic acid and ethyl alcohol. They also produced mannitol from fructose or sucrose and levulan or dextran from sucrose. The authors considered these properties justification for placing the organisms in one genus. They selected the name *Leuconostoc* because, as has been indicated, it had priority. They recognized three species: *L. mesenteroides* which fermented pentoses and sucrose, produced slime in sucrose solutions and was generally found in fermenting plant material and in sugar solutions; *Leuconostoc dextranicum* which fermented sucrose but not pen-

toses, produced a moderate amount of slime from sucrose, and may be associated with either plant or dairy products; and *Leuconostoc citrovorum* which failed to ferment either pentose or sucrose, produced no slime from sucrose, and was associated generally with milk or milk products. Several recent papers have considered the taxonomy of these organisms further (19, 20, 21, 58) and more than three species should probably be recognized. However, DNA-DNA hybridization studies also should be carried out with this genus.

In 1936, Matuszewski et al. (42) isolated and described citric acid fermenting streptococci different from the *Leuconostoc*. They grew at 10 C and 40 C but not at 45 C, survived 60 C for 30 min and produced dextro-rotatory lactic acid in milk along with copious quantities of carbon dioxide and C-4 compounds (diacetyl). They did not ferment glycerol, mannitol, xylose, sucrose, raffinose, or inulin, but produced acid from arabinose, dextrose, lactose, maltose, dextrin, and salicin. At about the same time van Beynum and Pette (3) described two citrate-utilizing organisms capable of producing lactic acid and diacetyl in milk. They used the name *Streptococcus citrophilus*. Swartling (68) studied many different strains of citric acid fermenting streptococci, and, from his results, suggested the organisms be regarded as *Streptococcus diacetilactis*.

Sherman (60) greatly clarified the status of *S. lactis* and *S. cremoris* by pointing out the clear physiological differences between them. The outstanding distinction shown between these two organisms was the ability of *S. lactis* to produce ammonia from peptone while *S. cremoris* was unable to effect this reaction. Later, Niven et al. (48) demonstrated that it was the amino acid arginine in peptone from which *S. lactis* liberated ammonia. Sherman did not comment on the statement of Orla-Jensen (49) that *S. cremoris* was the aroma-producing bacterium studied by Storch. It appears likely, however, that the organism studied by Storch was *S. diacetilactis*.

Krishnaswamy and Babel (39) suggested the names *Streptococcus lactis* var. *aromaticus* and *Streptococcus cremoris* var. *aromaticus* for *S. lactis* and *S. cremoris* strains able to produce small amounts of diacetyl. They differed from *S. diacetilactis* by not vigorously fermenting citric acid to produce large amounts of diacetyl.

No review of lactic acid streptococci would be complete without mentioning the serological investigations started by Lancefield (40) and extended by other workers. She developed a serological grouping method of differentiating the hemolytic streptococci. This method was applied by Sherman (61) to demonstrate conclusively that *S. lactis* was not a species in

any way related to *Streptococcus fecalis* which was in Lancefield group D. Mattick and Shattock (45) then described the preparation of a specific precipitating serum for *S. lactis* and established a new serological group of lactic streptococci to which the letter N was assigned. Briggs and Newland (7) applied their methods to *S. cremoris*, demonstrating that it also belonged in group N. This effort was shortly followed by the announcement of Briggs (8) that *S. diacetilactis* was also a member of serological group N, later confirmed by Sandine et al. (58).

RAW MILK

At one time, it was thought that freshly drawn milk was sterile. This idea was fostered by Lister (41) who in 1877, according to Harding and Wilson (25) presented a paper to the Pathological Society at London in which he made the following statement:

"In this case, the experiment was more rigorously conducted, and here, at first sight, you will suppose that no change at all had occurred, and in two of six weeks that there was no indication whatever of any organisms. I tapped one of them and found the milk still perfectly fluid, of normal taste and reaction and without any organisms in it, showing that unboiled milk, as coming from healthy cows, really has no fermentation in it capable of leading to lactic fermentation or any other fermentation or to any organic development whatever."

Thus on the basis of only two small samples, there was born the misconception that milk within a healthy udder was free of bacteria.

It was not until 1891 that Schulz, (59), cited by Harding and Wilson (25), found that fore-milk contained a large number of bacteria while those samples drawn later in the milking process contained fewer and fewer bacteria. Still the idea persisted for a number of years that normal milk drawn under aseptic conditions was sterile and that the presence of bacteria in milk was an indication of a diseased udder.

However, the work of Moore (44) in 1897 confirmed the findings of Schulz, and it was concluded that:

"Freshly drawn fore-milk contains a variable number of bacteria, varying in number from a few individuals to many thousands per cubic centimeter. These are distributed among several species. The last drawn at a regular milking contains, as compared with the fore-milk, very few microorganisms. It is the exception, however, to find a sample of milk free from microorganisms unless it is taken during the latter part of the milking process from a single quarter of the udder."

Once it was firmly established that bacteria do occur in normal raw milk, it was just a matter of time until the species of bacteria occurring in milk

would be brought under examination. One of the earliest studies in this regard was done by Walker (71). In his work, samples of fore and after milk were collected in sterile test tubes and gelatin plates were then made from these samples. Later, colonies were picked and subcultured on various differential media. It was found that *S. lactis* was the only species present which consistently occurred in all the milk samples; in fact, it was reported that this bacterium constituted at least 95% of the organisms present.

It is difficult to explain the results obtained by Walker now, since it is unusual to find streptococci of any kind making up 90% or more of the bacteria of milk samples. Perhaps he confused *S. lactis* with micrococci or other streptococci. Subsequently, in 1915, Sherman and Hastings (64) found streptococci in the milk of 31.1% of 48 cows and 15.1% of the samples from 161 cows.

Streptococcus cremoris is found in even lower numbers in milk. Of 3,000 isolates from 59 samples of commercial raw milk, only 4% were *S. cremoris*, according to a study made in 1952 by Nelson and Thornton (47). In that same year, Hirsch (now A. Hurst) (30) isolated 35 strains of lactic streptococci from raw milk samples in a remote area of the Jura Mountains in France. Two of the isolates were *S. cremoris*, adding credence to the idea that this species occurred in the wild state and was not a "mere environmental variety of *S. lactis*." In this study by Hurst, samples were collected from four producers that were about 80 miles apart, at an average altitude of 2,000 ft and that had never been in contact with laboratory produced starter cultures.

Recently, Radich (53) attempted to isolate lactic streptococci from producer raw milk supplies in the northwestern United States. When milk samples were held at 21 C for 48 to 72 hr before plating, isolates of all three species were made. However, most were *S. lactis*; of 31 producer samples tested, only 3 yielded *S. cremoris* isolates. Three different producer samples provided *S. diacetilactis* isolates.

THE UDDER INTERIOR

Since the lactic streptococci are found most often in milk, it was only natural that the bovine udder would be suspected as being a natural habitat for these microorganisms. One of the earliest investigations of this possibility was that done by Evans (16). Samples of milk were placed in sterile test tubes after part of the milk had been drawn to avoid contamination with bacteria, which might have entered the teat of the cow after the last milking. The samples were then plated out, colonies isolated, and identification tests conducted. From a total of 192 samples of milk studied, only one culture of *S. lactis* was isolated,

and this was discarded as a contaminant, as were all other cultures which were isolated only once. From the results of her experiment, Evans concluded that *S. lactis* did not localize and multiply in the udder.

The finding of Evans was confirmed by Rogers and Dahlberg (56). In their study, 29 samples of milk were obtained by milking directly into sterile test tubes. Ninety-three percent of the cultures isolated from these samples failed to reduce litmus. These cultures also were characterized by a general lack of fermentative ability.

SURFACE OF THE COW

The surface of the cow was at one time suspected as being a natural habitat for the lactic streptococci. Esten (15) was one of the first to investigate this possibility using cotton swabs to take samples. The first surface area studied was the inverted udder pocket formed by the flap of the skin attached to the hind leg and to the flank in front with the udder forming the back. The second was between the hip-bone and back bone and the third was at the union of the neck and shoulder. The cotton swabs were placed in sterile milk which was incubated at 21 C until the milk had curdled.

Samples of the curdled milk were then plated out on litmus lactose-gelatin agar and the organisms were identified to species by further tests. *Streptococcus lactis* was isolated from the surface of 21 cows out of a total of 24 animals tested. The organism was found most often in the udder pocket.

The ability of *S. lactis* to grow and multiply on the surface of a cow was doubted, and thus Esten (15) turned his attention to other sources of this organism. The cow's mouth was next investigated using the same methods. *Streptococcus lactis* was isolated from the mouths of 17 out of 21 cows. He postulated that the mouth provided an ideal medium for growth of these organisms because of the nutrients found there. Presence of *S. lactis* on the surface of the cow was believed to result from the cow licking herself.

In a later study done in 1921, Jones (33), upon isolating 35 strains of streptococci from the saliva of cows and others from the skin, came to the conclusion that "one feels justified in asserting that if *S. lactis* inhabits these regions, it exists in such small numbers that it cannot be detected. The souring of milk cannot therefore be attributed to the usual types found in the saliva, feces or vagina, or on the skin of cows." None of the cultures that he isolated appeared to have all of the characteristics attributed to *S. lactis*.

MILKING UTENSILS

It also was believed at one time that the lactic

streptococci gained entrance into milk via the milking utensils. Bergey (2) was one of the first to explore this possibility. Samples of milk were obtained from the milking bucket, strainer, and cooler; plated out; and developing colonies identified. Samples collected from the straining and cooling apparatus showed an increase in the number of bacteria present as the milk passed through the apparatus. It was believed that these organisms gained access to the milk either via air or through water used in washing the apparatus. However, in dairies in which all milking utensils and apparatus were sterilized prior to milking, the number of bacteria present differed very little from samples collected directly from cows.

As to the nature of the bacteria found in the milk samples, it was discovered that they primarily consisted of bacteria derived from the udder of cows, such as the lactic streptococci. In addition to these, a number of other species were found which had gained access into milk, and these were mainly types found in water.

The milker as a possible source of contamination was investigated by Esten (15). The surfaces of several parts of the human body were tested and no lactic streptococci were found. However, a large number of them were found in the human mouth, but it was felt that the original source of these organisms was the cow's milk.

FECAL MATERIAL

Due to inadequate and poor methods of identification, the lactic streptococci were reported to be a normal inhabitant of the intestinal tract of cattle. Presence of these organisms in fecal material was first claimed by Esten in 1909 (15). The lactic streptococci isolated from this source were found to be weaker in acid producing ability than those normally found in milk. This difference in acid production was attributed to the fact that *S. lactis* was probably a different variety from those normally found in milk; the passage of the organism from the mouth through the alimentary canal was thought to impair the vitality of the organism. While Esten used reducing action in litmus milk as the criterion for identifying *S. lactis*, this method would not differentiate the lactic streptococci from fecal streptococci.

Other instances of the supposed presence of *S. lactis* in fecal material are reported in the literature. McGuire (43) did a study on the percentage of *S. lactis* in fecal material. According to him, the relative number of these bacteria varied from four to 12% per sample. Heineman (28) compared the lactic streptococci he isolated from cow feces with those isolated from milk, separator slim, and teat wash water; he came to the conclusion that they all pro-

duced identical fermentations in milk.

Of the streptococci isolated by Ayers and Mudge (1) from feces, none were of the lactic group. Of 78 cultures of streptococci studied, 75 cultures did not grow in litmus milk at 10 C, no ammonia was produced from peptone, and on blood agar plates the colonies as a rule had a slight hemolytic zone. It was believed that these cultures were *Streptococcus bovis*.

Later isolation studies done on cow's feces such as those of Stark and Sherman (66) and Rogers and Dahlberg (56) confirmed the belief that cow's dung does not provide a natural habitat for the lactic streptococci.

SOIL

Soil as a possible habitat for the lactic streptococci has been suggested a few times in the literature. One of the earliest investigations of this possibility was that done by Esten (15). Twenty-four different types of soil were obtained, ranging from barren to the most fertile, from those found in swamps to those procured from highlands, and from those obtained in wooded areas to open fields. Strong gassy fermentations and a fragmented curd were observed when the soil was inoculated directly into milk tubes. The standard methods of isolation and identification were then carried out, but the results were always negative. Even samples of soil taken from a pasture on which 28 cows grazed showed no evidence of the presence of either *S. cremoris* or *S. lactis*.

Stark and Sherman's (66) attempt to isolate the lactic streptococci from soil was also unsuccessful. Their methods of isolation and identification appeared to be more precise than those of Esten in that tests were conducted on the ability of the isolated cultures to liquefy gelatin, produce ammonia from peptone, as well as carbon dioxide from glucose. An attempt was even made to isolate these organisms from the intestines of earthworms, but no positive results were obtained.

PLANTS

While dairy utensils are thought to be the most common immediate source of the lactic streptococci in milk, objects on which growth may occur do not represent a true habitat in an ecological sense. Likewise, the finding by several investigators of *S. lactis* in the bovine udder, mouth, and feces of cows was proven to be erroneous by later investigators who had more refined differential tests to identify the lactic streptococci.

By 1930, attention was being focused on plant material as the possible habitat of the lactic strepto-

cocci. One of the most fruitful investigations carried out along these lines was that done by Stark and Sherman (66). Samples of fresh and frozen corn, young corn silks, wheat flour middlings, dried navy beans, young cabbage heads, and many other vegetables were tested for presence of lactic streptococci. The plant material was first placed in sterile distilled water and held at room temperature for 24 hr. Gram stained preparations were then made from such infusions, and if the slide indicated the presence of Gram-positive cocci, the sample was plated out. Colonies were then picked and the standard differential tests were made on these pure cultures.

Two hundred cultures were isolated and identified as members of the lactic streptococcus group and these were all *S. lactis*. Samples of fresh corn and corn silks tested were found in every instance to contain *S. lactis*. This same organism was also isolated from frozen corn, navy beans, cabbage, lettuce, peas, and wheat middlings. *Streptococcus lactis* was not obtained from lima beans, alfalfa, cabbage, or mature corn.

The growth, fermentative, biochemical, and other characteristics of this group of organisms isolated from plant material was compared to those isolated from milk and milk products. In every instance the characteristics of *S. lactis* from plants agreed with those obtained from milk.

Not every investigator has found lactic streptococci on plant material as did Sherman. Esten (15) tried to isolate these organisms from grass, clover, and rye, but none was found. Leaves from trees, shrubs, and several garden plants were tested, but on none of these did the lactic streptococci occur.

Several different types of grain feeds were also tested, such as cottonseed meal, gluten, bran middlings, mixed feed, and corn meal. Only one culture of *S. lactis* was isolated from these sources, and that was from corn meal.

Results obtained by Pinter (52) in her study of the presence of streptococci on plant material differed from those of Esten (15). She did find *S. lactis* on grass and clover, as well as beans. In all, 50 samples of various plant materials were studied and, out of this number, 20% showed the presence of various streptococci. Out of the 20%, 70% were *S. fecalis* and 30% were *S. lactis*.

Attempts to isolate *S. cremoris* from plant material have been made by a few investigators, but with little success. Yawger (73) tried to isolate this organism from over 60 samples of plants. No strains of *S. cremoris* were found, but he did manage to isolate 16 cultures of *S. lactis*. He felt that even though the results of his experiments were negative, plant materials still presented the most logical source

for *S. cremoris*. In 1965, Cavett, et al. (10) reported isolating strains of *S. cremoris* from frozen peas. Cavett and Garvie subsequently indicated (11) that these cultures were incorrectly designated and were, in fact, unusual group N streptococci with properties different from both *S. lactis* and *S. cremoris*.

Although *S. diacetilactis* was originally isolated from plant material (42), later investigators such as Swartling (68) obtained this organism from dairy starters or dairy products. This suggests that the organism occurs in nature associated with plant material and enters raw milk from this source. It is not likely that this bacterium is a mutant of *S. lactis*, but the possibility that *S. lactis* strains are variants of *S. diacetilactis* that have lost the ability to ferment citric acid is possible. In this regard, it is noteworthy that citrate permease negative strains of *S. diacetilactis* have been described by Harvey and Collins (27).

In 1968 Radich (53) also surveyed fruits and vegetables for occurrence of lactic streptococci. Twenty-seven different species of vegetables and 18 species of fruits were examined. Only *S. lactis* was found in low numbers on potatoes, corn, cucumbers, peas, beans, and cantaloupe. None of the other vegetables or any of the fruit yielded the organisms sought. Even more recently, King and Koburger (35) characterized Group N streptococci isolated from meats, frozen vegetables, dairy products, barn-trough water, and poultry feed. From 18 samples, 184 isolations of *S. lactis* were made which were generally more resistant (94.6%) to 20 bacteriophages than dairy starter culture isolates (77% resistant). No *S. diacetilactis* strains were isolated and *S. cremoris* was recovered from only cottage cheese and raw milk. Unfortunately, the *S. cremoris* found in milk were lost because they failed to survive freezing.

The natural habitat for *S. cremoris* still remains unknown, and more studies similar to that of Hirsch (31) are needed to understand its ecological relationship to other streptococci. This is especially true since new strains are needed in industrial fermentations. The possibility that this organism is present in the same environment as *S. lactis* but in lower numbers is attractive but unproven. If such is true, isolation by dilution is impossible and a new plating method allowing rescue of *S. cremoris* is needed.

ACKNOWLEDGMENT

The authors acknowledge the interest and advise of Erik Lundstedt in reviewing the manuscript and locating some of the older references cited.

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ASSOCIATION AFFAIRS

NOMINATIONS FOR OFFICES OF IAMFES, INC.—1972-1973

(Notice to membership—ballots can only be mailed to paid up members as of April 15, 1972)

FOR SECOND VICE-PRESIDENT AND SECRETARY-TREASURER



EUGENE T. MCGARRAHAN

Eugene T. McGarrahan is a Food Technologist and Assistant to the Chief of the Dairy, Fats and Oils Branch of the Division of Food Technology, Office of Product Technology, Bureau of Foods, of the Food and Drug Administration, Washington, D. C.

By working on several dairy farms and an independently owned milk bottling plant he was inspired to enroll in dairy technology at the University of Illinois, where he received a Bachelor of Science degree.

While in military service he taught Chemical, Biological and Radiological Warfare courses to infantry units and participated in the 1952 field exercises on radiation detection and decontamination at the Atomic Energy Commission proving grounds, Camp Desert Rock, Nevada.

After graduating from the University of Illinois he worked for private industry in various aspects of manufacturing and distributing dairy products. In 1961 he began his Civil Service career with the Dairy Division of the U. S. Department of Agriculture. Initially he was assigned to the Inspection and Grading Branch where he performed plant and product inspections, graded products, and later as a resident plant inspector also performed laboratory analysis of milk and milk products. In 1963 he transferred to Washington, D. C. where he worked for the Dairy Standardization Branch in the area of developing grade standards, product specifications and milk and milk quality improvement programs. In 1966 he transferred to the Foreign Agricultural Service of USDA and worked for the Dairy and Poultry Division's Foreign Marketing Branch. In 1968 he transferred back to the Dairy Division and became the Assistant Chief of the Standardization Branch and served in that position until June of 1971.

During his career as a Federal employee he has served on 3-A ad-hoc committees and later on the I.A.M.F.E.S. Committee on Sanitary Procedures for the purpose of assisting in the development of 3-A Sanitary Standards for food equipment. Since 1967 he has served as a member of a U. S. interagency work group on international dairy products standards, and has attended several of the annual sessions of the FAO/WHO Committee of Government Experts on Milk and Milk Products, as a member of the U. S. Delegation. From 1967 to the present time he has been a member of the American Dairy Science Association's Dairy Product Evaluation Committee.

Mr. and Mrs. McGarrahan reside in Sterling Park, Virginia and have three children who are actively engaged in Loudoun County 4-H activities.



HAROLD E. THOMPSON, JR.

Harold or "Tommy," a native of Massachusetts, graduated from the University of Maine in 1941 with a B.S. degree in Dairy Technology. Following graduation, he spent a year as assistant superintendent of production in an ice cream plant before entering the army in 1942, where he spent the next four years as an officer in the Medical Corps.

In 1946, he joined the Virginia State Department of Health as a county sanitarian conducting programs in all phases of environmental health. The following year he became Assistant State Milk Sanitarian with the Virginia State Department of Health with responsibilities in the implementation of a Statewide milk sanitation program.

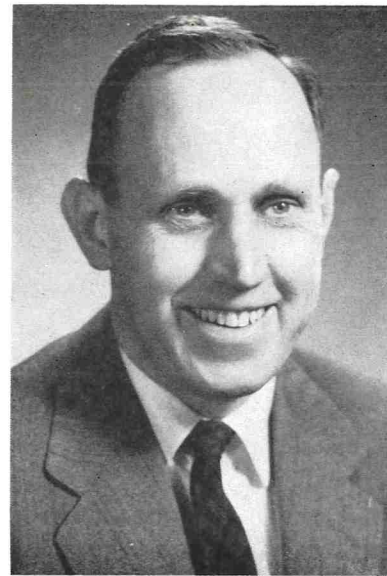
In 1949 he was commissioned by the U. S. Public Health Service and entered on duty as Regional Milk and Food Consultant in the Region I office in Boston. He served in the New York office and Washington, D. C. until attending the University of Minnesota in 1959 where he received a Master of Public Health Degree. On completion of his graduate studies, he was transferred to the Kansas City regional office, remaining there until accepting the position of Chief, Milk Sanitation Program of the United States Public Health Service.

In 1970 the U. S. Public Health Service awarded him the Commendation Medal for sustained high quality work performance and noteworthy technical and professional contributions in the scientific and administrative fields of public health.

He has actively promoted milk and food sanitation as a member of the International Association of Milk, Food and Environmental Sanitarians as well as serving on the Board of Directors of the National Mas-

titis Council, the Executive Committee of the National Conference of Interstate Milk Shipments, the Steering Committee of the 3-A Sanitary Standards Committees and the Joint FAO/WHO Committee of Governmental Experts on the Code of Principles Concerning Milk and Milk Products.

SECRETARY-TREASURER



RICHARD PELL MARCH

Richard P. March is a professor in the Department of Food Science at the New York State College of Agriculture, Cornell University, Ithaca, New York. Until 1965, he devoted 75% of his time to extension work as a specialist in milk quality and fluid milk handling and processing, and the balance of his time in research and teaching courses in fluid milk processing and quality control. At present, extension accounts for 90% of his time with 10% for research activities.

He was raised in Massachusetts, majored in dairy industry at the University of Massachusetts, receiving a B. S. degree in 1944. After a tour in the U. S. Marine Corps, he entered the Graduate School at Cornell University to major in dairy industry, receiving an M. S. degree in 1948.

Professor March taught a one-year program in dairy manufacturing until its termination in 1951, at which time he was promoted from instructor to assistant professor. He became an associate professor in 1955, and full professor in 1965. In 1965 he also became department extension leader and is still serving in this capacity.

He is active in the New York State Association of Milk and Food Sanitarians, serving as secretary-treasurer from 1957 and executive secretary since

1967, secretary of the Dairy Industry Equipment Committee from 1952-57, secretary of the Farm Practices Committee from 1955-62, and secretary of the Council of Affiliates since 1952. He is a member of the International Association of Milk, Food, and Environmental Sanitarians, a member of their Farm Methods Committee from 1959-65, secretary of their Council of Affiliates in 1961, and chairman in 1962 and 1963.

In both the State and International Associations he has served as chairman of a number of subcommittees including the Uniform Milkhouse Plans for the Northeast, Milk Transfer Systems, Sediment Testing, and Training Programs for Bulk Tank Truck Operators, and co-chairman of the Northeast Committee on Uniform Guidelines for Loose Housing Systems. In 1963 he was the recipient of the New York State Association's Dr. Paul B. Brooks Memorial Award for outstanding contributions to the organization.

HYDROGEOLOGY

OF SOLID WASTE DISPOSAL SITES IN NORTHEASTERN ILLINOIS

Final Report on Hydrogeologic Study of Solid Waste Disposal Sites in Illinois

Hydrogeology of solid waste disposal sites in northeastern Illinois; a final report on a solid waste demonstration grant project. G. M. Hughes, R. A. Landon, and R. N. Farvolden. Washington, U. S. Government Printing Office, 1971. 154 p.

One of the problems inherent in disposing of refuse on land is the everpresent danger that, unless properly engineered in a sanitary landfill, the wastes will adversely affect groundwater resources.

This problem was investigated in a study supported by the U. S. Environmental Protection Agency under one of the demonstration grants authorized by the 1965 Solid Waste Disposal Act. The study was conducted by personnel of the Illinois State Geological Survey and was sponsored by the Survey, the Illinois Department of Public Health, and the University of Illinois at Urbana. Although the initial objective was to obtain hydrogeologic information about landfills, it was apparent after the first two years of work that a considerable amount of precise data on water quality could be gathered with relatively little effort or expense. This data gathering was emphasized during the final year of the project. The conclusions reported apply specifically to the soil types that were tested, but the procedures and methods used for the testing are applicable for future hydrogeologic-landfill research.

Single copies of this report are available without

charge from solid waste management publications distribution, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268. Multiple copies (at \$1.50 per copy) are for sale by the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402.

NORMAN N. TOBEY

Norman N. Tobey, St. Petersburg, Florida was stricken with a massive cerebral hemorrhage on February 1, 1972 while at home.

Norman was 48 years old, born and educated in Maine. He graduated from a business college in Portland after serving in the Navy during World War II. He joined Taylor Instrument Co., Rochester, N. Y. in March 1953. He was assigned to Atlanta, Georgia and then to St. Petersburg for the past 12 years.

Norm was an outgoing person, highly competent, with an engaging personality. He was a member of the Instrument Society of America, Past President of the Florida Association of Milk, Food and Environmental Sanitarians and a member of International Association of Milk, Food and Environmental Sanitarians, Inc. He served as general chairman of the International Association's annual meeting at Miami Beach in 1967. He received the "Outstanding Industry Man of the Year Award," at the Florida Association Annual Meeting in March 1971.

Norman will be greatly missed by all his friends and associates. The members of the Florida and International Association extend their deepest sympathy to his wife Priscilla and to their daughters Tracy, Beth and Gayle.

RUDY ZELM

It is with great regret that we announce the death on February 17 of Rudy Zelm, long-time representative of the American Dry Milk Institute on the Sanitary Standards Subcommittee - Dairy Industry Committee. A letter from Dr. Warren Clark, of the ADMI staff, dated February 18, advises that Rudy suffered a heart attack on February 14 and was hospitalized immediately, but did not recover.

Rudy was a personal friend of many in the 3-A committees, having participated in the 3-A work for at least ten years. During part of this period he was a member of the 3-A Symbol Administrative Council, but for the greater part of these years Rudy represented the user group for dry milk equipment on the SSS.

Only as recently as January 26 Rudy met with the DFISA Task Committee, in a counseling capacity, for the consideration of sanitary criteria for bulk

powder handling equipment. His contribution at that time was pivotal in the determination of problem areas for this equipment.

Rudy will indeed be missed at meetings of the 3-A committees.

CARPET IN COW BARN INCREASES MILK PRODUCTION

Some 160 cows have been giving eight to ten pounds more milk each per day since David Heimerdeinger installed Ozite carpeting in his Hickory Dairy Farms in Clinton, Michigan, six months ago. Heimerdeinger reports this represents \$1,500 more milk per month.

Bovine aesthetics were not Heimerdeinger's chief consideration when he decided to install the 300 square yards of blue gray New Vision carpet in his \$175,000 cow barn. He says he's saving \$1,200 per year in straw bedding, as the Holsteins sleep on the carpet.

Free stalls in the barn are situated one foot above a concrete floor equipped with special manure pits using mechanical cleaners. The cows are encouraged by an electric trainer to make their deposits in the alley to the rear of their stalls rather than on the carpet, said Heimerdeinger. He indicated this is easy to accomplish since cows have a natural tendency to back up for defecation anyway.

"We used Ozite's water proof glue to anchor the carpet," he explained, "so cleaning up is simply a matter of hosing down and brushing." Ozite Corporation, Libertyville, Ill.

NEW MULTI-STAGE HIGH PRESSURE CLEANING SYSTEM

A new type central system introduced by Klenzade offers separate automatic high pressure pump staging to meet plant multiple drop station flow requirements. The working pressure of the system is 600 psi at 40 gpm. It is completely automatic from shut-down at no flow to start-up when one drop station is in use and additional pumps engage as more stations are utilized. Flow rates and pressure are adjustable. This system has flexibility with standby pump capability. It is available in one to any number of compact pump modules, preassembled with ready-to-connect pipe manifolds. Pumps are three cylinder stainless steel with chrome plated heads. Standard drop stations have 40 foot 1125 psi dual hose for chemical and hot water with shut-offs at spray wand. All systems tailored for specific plant applications.

Write Klenzade Products, Division of Economics

Laboratory, Inc., Osborn Building, St. Paul, Minnesota 55102 for descriptive literature.

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CLASSIFIED ADS

FOR SALE

Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology Inc., P. O. Box 101, Eugene, Oregon 97401.

MANAGER-SANITATION CONTROL

Large manufacturing division located on East Coast requires Manager to direct inspection function. Previous experience with either private or governmental agencies desired; position reports to Director of Manufacturing.

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Micron Generation*

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The Micron Generating process utilized in the small Micro-Gen system and companion medium and large units combines:

Carepower—Micron Generation permits economic use of higher grade chemical insecticides such as the high concentrate pyrethrins which offer high "flush" and "kill" impact on insects but are the safest of chemicals to use in terms of environmental effect on vegetation, wildlife and humans, and

Killpower—Micro-Gen units have been proven the deadliest on the market, with professional pest control operators reporting body counts of up to ten times that achieved with standard equipment used immediately before in restaurants, food processing facilities, warehouses and similar establishments.

Micron Generation is Micro-Gen's proprietary process for dispersing insecticides and other chemicals in liquid droplets measuring from one to fifteen microns (millionths of a meter) in diameter. This compares with the 100 microns plus size of droplets produced with standard spray equipment. The much smaller droplet size makes possible intense, uniform penetration of infected areas, reaching hidden nests and runways as it permeates virtually all locations that air itself can reach. By-product pollution is avoided because the Micron Generation process relies on direct impact of droplets upon target insects, not on contact with a sticky "residual" coating as is utilized in most insecticide treatments. Micro-Gen Corp.

*Trademark of Micro-Gen Corp.

supplies a full line of chemical agents structured to take optimum advantage of the Micron Generation capabilities of its equipment. One of the most important of these is BP 300 insecticide, a distillate of botanical pyrethrin which is especially effective at structural pest control.

For food processing and food handling companies, for bottlers, brewers and bakers, for warehouse and storage elevator operators, for apartment and housing project operators, and for many other insect-vulnerable operations, Micro-Gen equipment and chemicals constitute a powerful new weapon in the struggle against insect damage—and in the concurrent struggle to avoid contamination of the environment through the use of possibly harmful insecticides.

For further information on Micro-Gen equipment and on the combined "Killpower / Carepower" benefits in pest control it can provide to your organization, contact your local pest control operator.



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Albuquerque, New Mexico 87110 (505) 266-4140

Dairy authorities speak out
on better cow milking.



V. L. Baldwin/Virginia Polytechnic Institute
and State University/Blacksburg

Understanding between man and cow can mean more milk

Some dairymen and milkers consistently get more milk out of cows because they understand and take advantage of the animals hormone functions. In fact, they get so much more milk that dairy experts suggest others follow their example.

At milking time, if a cow is not stimulated to let down her milk, her production will suffer. If fear, pain or disturbances occur, she will not produce as much. A creature of habit, she responds to procedures which are repeated day after day.

While milk let down must be fully stimulated, dairymen must remember that the stimulation lasts for only an average of six or seven minutes. The entire job of milking each cow must be completed within that time period. Good practice dictates that the milking unit should be placed on the cow very soon after milk is let down.

To get all the milk, the milker needs the cows full cooperation. High producing cows and those with tight sphincter muscles will take more time so the milking routine should take these animals into consideration.

Most cows are actually milked out in two to five minutes after the milk has been let down. Capable dairymen observe milk flow changes. When the flow slows down because most of the milk is removed, gently pull forward and down on the milking machine. This action along with gentle massaging of the individual quarters will help release trapped milk.

Overmilking causes irritation and stress. It creates conditions which could result in mastitis. Overmilking also tends to produce slow-milking cows. They begin to expect pain and at a certain point secrete the "interference" hormone, adrenalin, which prevents rapid milk let down. Many of

the hard-to-milk cows will even gradually change their habits if the milker will change his and encourage fast milking.

Best results occur when the milker limits the number of units he operates. His rule should be, no more than two units in a milking barn, and only three in a milking parlor. Only with such new aids as prep-stalls and automatic removal can one man successfully operate more machines.



Proper milking procedures are taught
in Virginia Tech milking schools.

EFFICIENT EQUIPMENT IMPORTANT

Perfect machine operation cannot compensate for inadequate or improperly adjusted equipment. Inadequate air flow may result from a pump that is worn or too small, vacuum or milk lines which are too small, too long, not sloped enough, plugged, or having other restrictions including filters or flooding with milk, or from excessive leaks. The equipment representative can check these things. It is to your advantage to let him install and maintain a fully adequate system which will equal or exceed 3-A Accepted Practices recommendations.

Some simple checks can be

helpful when inadequate capacity is suspected. For example, when all components using air are attached, a petcock may be opened to reduce the vacuum level to 10" instead of 15". Then close the petcock and count 1000, 2000, etc. If it takes more than 2 seconds to recover to 15", the air flow capacity may be too low.

Vacuum (or air removal) actually operates the machine and takes milk from the cow. Air flow, measured in cubic feet per minute (C.F.M.), from both vacuum and milk pipelines is necessary to maintain the recommended vacuum level.

Air flow capacity of a pump and a system can be measured by an air flow meter. There should be no more than a 10% loss between the pump and the system air flow. Your equipment dealer should provide for and explain such things as the need for a reserve tank, traps, cleaning of vacuum lines, limiting vacuum fluctuations, keeping vacuum gauges, regulators, pulsators, pumps, etc. functioning properly.

Milking speed will tend to increase with increased vacuum level and pulsation ratio (milking-massage ratio). Possibility of irritation to the udder also increases accordingly. While milk is flowing it cushions irritations. A good milker will see that the machine is not attached to the cow when milk is not flowing. He will prepare the cow by using a strip cup to detect abnormal milk and remove bacteria from the teat end, then wash and dry the udder with a single service towel and attach the machine. He will keep his hands and the milking unit sanitized. When each quarter milks out, remove teat cups promptly and dip the teats in a specially prepared teat dip.

Súrge... the accent is on YOU!

This is one of a series
of topics developed by
noted Dairy authorities.
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