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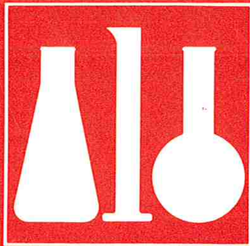

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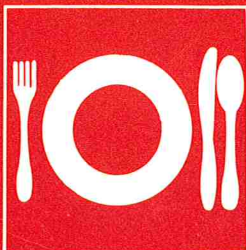

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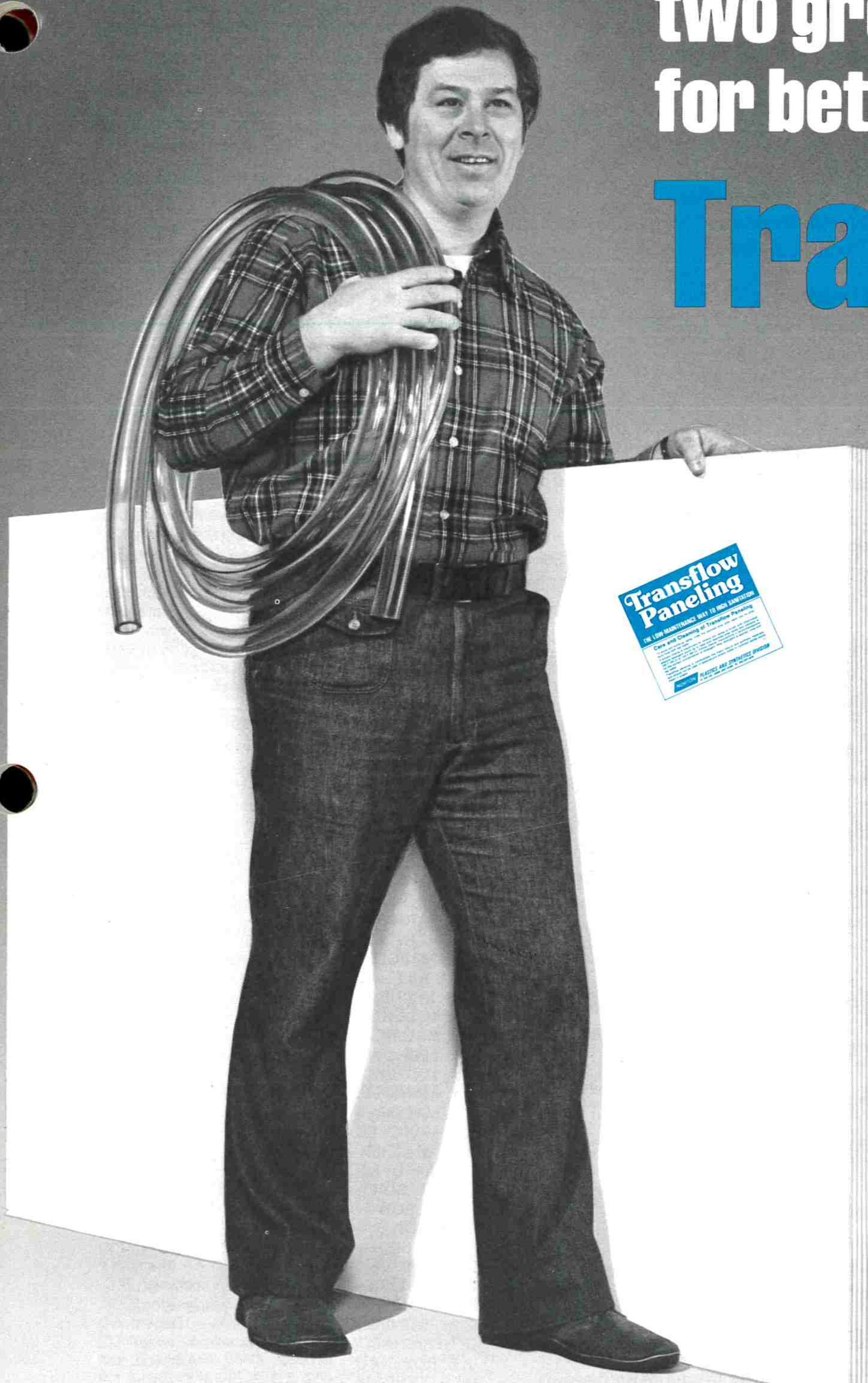

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Growth, Sporulation and Aflatoxin Production by *Aspergillus parasiticus* on Strained Baby Foods

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ABSTRACT

The potential for aflatoxin production by *Aspergillus parasiticus* on strained baby food was evaluated. Four puréed foods were inoculated with the mold and cultured at 15 and 26 C in two series of experiments. The aflatoxigenic mold produced mycelia and sporulated at both temperatures. The foods ranked in mean total yield of aflatoxin (μg/g of substrate) in the following order: peas > squash > green beans > pears. The ranking held consistent for both temperatures. Aflatoxins B_1 and G_1 were produced in higher percentages than B_2 and G_2 in each food at both temperatures. At 26 C, total aflatoxin produced ranged from 8 to 71 μg/g of substrate, and at 15 C, the mean for the four foods was from 3 to 50 μg/g of substrate. Temperature and substrate were the primary variables which contributed to sporulation rate, toxin production and toxin ratios. Peas and squash should be considered primary and highly supportive substrates for aflatoxin production if conditions should arise for spores to contaminate the products either during or after processing. Absolute prevention of aflatoxigenic spore contamination in these foods studied is essential. An occasional testing of these foods for aflatoxin seems warranted. A lower temperature during aflatoxin formation decreased the total toxin formed, but did not prohibit aflatoxin occurrence. A lower temperature also tended to divert the type of toxin produced from B_1 to the less dangerous G_1 and G_2. Aflatoxin would appear to be a problem in these foods only under rare and unusual circumstances in relation to processing and consumer usage. If such aflatoxigenic spore contamination should occur, the levels produced would be significant.

Molds and fungi are very common in our environment. Some seem harmless while others may produce metabolites of a pathogenic nature (*9,18*). Increased interest in mold metabolites or mycotoxins was aroused in 1960 due to the "Turkey X Disease" epidemic found to have been caused by metabolites of the mold *Aspergillus flavus* (*19*). Since then, numerous studies have been conducted to evaluate the disease-producing potential of such mycotoxins.

The most potent disease-producing mycotoxins are aflatoxins (AFT), a group produced principally by *A. flavus* and *Aspergillus parasiticus* (*3,5*). Aflatoxins have been found to produce toxic and carcinogenic effects in fish, birds and mammals (*16*). Further details of the chemical, biological, toxicological and clinical features of identified aflatoxins may be found in review articles by Moreau (*15*), Wright (*24*), Goldblatt (*8*), Wogan (*23*) and Ciegler and Lillehoj (*4*).

There are a variety of substrates which allow fungal growth and aflatoxin production under natural and laboratory conditions. Sorghum, peanuts, corn, wheat, rice (*10*) and soya-beans (*2*) seem particularly favorable. These and other agricultural products have been examined in the field, at harvest time and in storage in an effort to determine which situation(s) and their particular set of variables is most conducive to mold growth and toxin production (*11*). Natural foodstuffs, as well as those which have been processed, have proven susceptible. Grapes, peaches, cheese, fruit juices (*22*), walnuts, condensed milk, beans and figs (*7*) are some which have shown mold growth and aflatoxin production in experimental conditions. Coconut supports aflatoxin production particularly well and has been used as a growth medium for laboratory studies (*1*). Tobacco is an unusual exception in that the fungus does grow and produce toxin in nature, but aflatoxin does not survive the burning process and has not been found in the smoke of cigars and cigarettes (*17*).

The occurrence of aflatoxin-producing molds in foods should be a particular concern to the consumer. Because aflatoxins are capable of producing toxic and carcinogenic effects in a number of animal species, it seems likely that a threat to man exists. In 1977, the FDA ordered a recall of mold-contaminated (*Penicillium*) baby foods which brought attention to this likelihood (FDA recall letter and news release statements, June 3, 1977). The occurrence of potential toxin-producing molds in baby foods should be of special concern as it is known that sensitivity to aflatoxins is greater in the younger members of a species.

With this in mind, this study considered four types of baby foods in an effort to determine their potential to support mold growth and aflatoxin production at room and refrigeration temperatures.

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MATERIALS AND METHODS

Culture preparation

Twenty-eight 500-ml flasks were used for each of the two series of experiments. Each flask contained 10 ml of water and was autoclaved for 20 min (125 C, 20 psi). After sterilization, the water was decanted from each flask and 10 g of each baby food were added under aseptic conditions. The flasks were then sealed with sterile cotton plugs.

Baby foods from two major U.S. companies were used. These included strained pears, strained squash, strained peas and strained green beans. None of these foods contained salt or sugar additives, preservatives, flavor enhancers, artificial colors or flavors. The pears did, however, contain citric acid and vitamin C in addition to the strained fruit.

Inoculation

A. parasitcus NRRL 2999 culture grown on potato-dextrose-agar slants for 3 weeks, in darkness, at room temperature was used to produce the spores to inoculate each baby food sample. One loop-full of inoculum containing approximate equal portions of spores was placed into each culture, using aseptic inoculation techniques. Experimental flasks were made in triplicate for each food tested. Control flasks, also in triplicate, were not inoculated with the mold. After inoculation, the baby food samples were cultured for 12 days. Series I was maintained at room temperature (26 ± 3 C) and a relative humidity of 70 ± 5%. Series II was cultured at 15 ± 1 C and 80 ± 3% relative humidity in an environmental chamber.

Extraction and analysis

Cultures were attenuated by adding 40 ml of $CHCl_3$ through the cotton plug with a 50-ml syringe and needle. A chloroform and water extraction procedure following the A.O.A.C. format was used *(12)*. Portions of the extracted solutions and dilutions were tested for the level of aflatoxin present by using thin layer chromatography (TLC). Visual determination for aflatoxin concentrations was made by comparing fluorescent spots to reference samples *(12)*. All toxin analyses were completed at the Mycotoxin Laboratory of the Virginia Division of Consolidated Laboratory Services, Richmond, Virginia.

RESULTS

Fungal growth and sporulation

For Series I, grown at 26 C, growth was observed as white halos around the inoculum in squash and green beans after 24 h. At this time, there was no evidence of mycelial growth in the peas and pears. By 48 h, mycelia covered all cultures and sporulation had begun in squash and peas. Sporulation increased in all cultures after 48 h. Controls showed no growth or sporulation. Room temperatures during these observations ranged from 24.5 to 28 C; relative humidity ranged from 68% to 77%. For Series II, grown at 15 C, fungal growth appeared as small white halos in all cultures except pears by 72 h. Growth occurred in pears by day 5. Mycelial growth increased in all cultures and by day 10 sporulation had begun in the green beans and pears. Control cultures remained clear. Cultures in Series II were maintained at 15 C and a relative humidity of 80 ± 3%.

Toxin production

Aflatoxins were detected in various amounts in all inoculated cultures at both 26 and 15 C. Control cultures showed no trace of toxin (see Table 1).

DISCUSSION

The four foods were ranked according to mean total yield of aflatoxin as follows: peas > squash > green beans > pears. The overall ranking was consistent for both temperatures. Based on the new Duncan's multiple range test ($p \leqslant 0.05$), the total levels of aflatoxins showed significant differences between all four substrates at the lower temperature except for squash vs. green beans. For the higher temperature treatment, only the green bean comparison to pears failed to be significantly different. When high and low treatments for the same food substrates are compared, each substrate tested yielded significantly less total aflatoxin at the lower temperature than the same substrate and mold grown at the higher temperature. This response indicates the significance of both the temperature at which the mold grew and probably the chemical differences in the substrates. Studies have shown that certain vitamins and especially zinc levels in the substrate will affect growth and toxin production *(6)*. It is doubtful that the low production of toxin was due to inhibitory factors present in the foods.

TABLE 1. *Aflatoxin production in μg/g of substrate for four foods inoculated with* A. parasiticus *and grown for 12 days at 15 C and 26 C.*

	B_1		B_2		G_1		G_2		Total	
Food substrates	26 C	15 C	26 C	15 C	26 C	15 C	26 C	15 C	26 C	15 C
Peas	29.63[a]	11.75	—	—	40.63	37.85	1.21	0.31	71.48	49.91
	± 4.70	± 1.06	—	—	± 9.35	± 11.38	± 0.32	± 0.31	± 14.33	± 10.63
% of total	41.5	23.5			56.8	75.8	1.7	0.6		
Squash	20.80	2.92	0.38	0.35	15.57	2.29	0.29	0.58	36.79	6.14
	± 9.21	± 1.44	± 0.00	± 0.05	± 8.72	± 0.96	± 0.09	± 0.15	± 18.13	± 2.21
% of total	56.3	47.6	1.0	5.7	42.0	37.3	0.7	9.4		
Green Beans	2.50	0.50	0.26	0.08	6.05	4.07	0.59	0.47	9.40	5.11
	± 1.88	± 0.06	± 0.13	± 0.01	± 0.97	± 0.45	± 0.16	± 0.13	± 2.39	± 0.64
% of total	26.6	9.8	2.7	1.4	64.4	79.6	6.3	9.2		
Pears	5.21	1.46	0.20	0.44	2.29	1.05	0.11	0.32	7.81	3.27
	± 1.30	± 0.36	± 0.03	± 10.39	± 0.36	± 0.35	± 0.04	± 0.05	± 1.66	± 0.83
% of total	66.7	44.6	2.6	13.5	29.3	32.1	1.4	9.8		

[a]Mean yield and standard deviation in μg of toxin/g of substrate.

Temperature was a significant factor in the formation of aflatoxin in these foods. The mean total aflatoxin yield was consistently highest at 26 C for each food. This observation agrees with the findings which indicate that the optimal growth temperature for *A. flavus* is near 24 C (*20*). The yields of individual aflatoxins (B_1, B_2, G_1, G_2) often vary with temperature. However, the two most potent, B_1 and G_1 have been found to occur at higher levels than B_2 and G_2 at both high and low temperatures (*20*). The foods tested here showed a greater percentage of B_1 at the higher temperature; G_1, B_2 and G_2 generally occurred in higher percentages at the lower temperature. When B_1 levels are compared to G_1 produced at the two temperatures on all four foods, it becomes apparent that at reduced temperatures the percentage of B_1 decreased in all foods. Concurrently, the percentage of G_1 increased in three of the four foods. The nutrient content of these foods may have been responsible for the increased yield of G_1 at lower temperatures. No B_2 was formed in the peas at either temperature. For this strain, B_2 is only detected in the presence of very high levels of B_1.

The consistently higher yields of B_1 and G_1 at both temperatures for each food indicates the need to consider foods during their processing when they may be accidently maintained for long periods at temperatures optimal for the growth of *A. flavus*. Although this occurrence is highly unlikely, several studies have indicated the potential danger of toxin production at both high and low temperatures. Van Walbeck et al. (*21*) found that even temperatures from 7.5 to 10 C (a common range in domestic refrigerators) could indeed be conducive to growth and toxin production in foods kept for any length of time. Our growth studies at 15 C support this also. Slight refrigeration seems to have two advantages: that of reducing the total aflatoxin produced and diverting of the type of toxin produced to less toxic G_1 or G_2. On the other hand, a study by Meir and Marth (*14*) indicated that storing sausage or other cured meats at high temperatures for some time would not absolutely prevent growth and toxin production. To prevent aflatoxin accumulation, it seems necessary to establish ranges of temperature and lengths of time for the safe storing of food. Processing techniques should be considered as well since some additives used in foods, such as sugar or zinc, seem conducive at certain levels to the growth of *A. flavus* and toxin production.

Baby foods contaminated with mold and requested to be withdrawn by the FDA were probably contaminated during processing by spores remaining from a previously processed food. Since it is common practice and economical to use cleaned processing equipment for a variety of foods, such occurrences of carryover contamination, although not common, hold potential danger for many foods, especially peas and squash. The problem becomes evident when the spores carried over flourish in the new substrate. Machinery slow down, break-down, or shut-down with or without proper cleaning and refrigeration could allow low levels of spore contamination to develop into a more serious problem. Generally this would occur only under the rarest of circumstances. Subsequent heat treatment would eventually destroy the organism and the spores but most of the toxin would remain if any had developed.

Obviously, the best approach would be absolute elimination of spore contamination carryover from foods previously processed using the same equipment or the spores from foods currently being processed. Even the chronological order of food processing should be considered. Of the four foods tested, peas hold the greatest potential for aflatoxin production. It is suggested that additional caution be exercised to reduce the carryover spore contamination risk for this food. Also, it is evident that mild refrigeration, even at 15 C, reduces the mold growth and aflatoxin production substantially but not absolutely. The lower temperature also tends to favor the occurrence of less dangerous G_1 and G_2 over B_1. Improper storage of opened baby foods in the home probably would be more conducive to time/temperature relationships suitable for aflatoxin production than those existing in the processing procedures.

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Survival and Growth of *Clostridium* Species in the Presence of Hydrogen Peroxide

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ABSTRACT

Some bacteria in the genus *Clostridium* can occur as contaminants in milk. If cheese is made from milk with such contaminants, the bacteria can cause the "late gas" or "late blowing" defect in the cheese. Since hydrogen peroxide can be used to treat milk for cheesemaking, this investigation was initiated to determine effects of the peroxide on viability and growth of *Clostridium tyrobutyricum* NIZO, *C. tyrobutyricum* 144, *Clostridium perfringens* 115 and *Clostridium sporogenes* T9. Presence of 0.01% hydrogen peroxide in litmus milk retarded but did not prevent growth of and gas production by the clostridia. Presence of 0.02% peroxide inhibited growth and gas production when litmus milk contained, per milliliter, 50 or 100 spores of any of the clostridia being studied. These numbers of clostridial spores are greater than those normally found in raw milk produced under ordinary conditions.

Some of the microorganisms present in milk play an important role in causing defects in dairy products. Bacteria of the genus *Clostridium* present a special problem to the cheesemaker. The "late gas" or "late blowing" defect usually occurs in hard cheese made from milk contaminated with anaerobic sporeforming rods of the genus *Clostridium*, particularly *Clostridium tyrobutyricum* (*3,9*). This defect results from production of gas which causes a bloated cheese with a spongy consistency. The bloated condition is always accompanied by formation of various unacceptable flavors which appear in contaminated cheese a few weeks after manufacture, depending on the number of spores and the species of *Clostridium* present (*4*).

Hydrogen peroxide (H_2O_2) has been used in the dairy industry for two purposes: (*a*) as a preservative for raw milk before processing, and (*b*) in combination with a heat treatment to reduce the total bacterial count of milk before cheesemaking. Such use of hydrogen peroxide has been greatest in tropical or sub-tropical countries, although some cheese in U.S. is made from milk treated with hydrogen peroxide. The first experimental use of hydrogen peroxide as a preservative for milk was reported in 1883 (*7*). Although hydrogen peroxide can be used for cheesemaking, the effect of different concentrations of H_2O_2 on viability of spores of *Clostridium* species, the main cause of the "late blowing" defect in cheese, has received only limited attention from research workers. Accordingly, this investigation was initiated to determine effects of the peroxide on viability and growth of *Clostridium tyrobutyricum* NIZO, *Clostridium tyrobutyricum* 144, *Clostridium perfringens* 115 and *Clostridium sporogenes* T9.

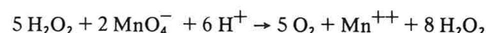
MATERIALS AND METHODS

Cultures

A culture of *C. tyrobutyricum* NIZO was obtained from Dr. Galesloot at the Netherlands Institute for Dairy Research, whereas *C. tyrobutyricum* 144, *C. perfringens* 115 and *C. sporogenes* T9 were supplied by the Department of Dairy Microbiology, Institute of Dairy Technology, IPM, Warsaw, Poland. Growth and sporulation of these cultures were done as was described by El-Gendy (*4*), using litmus milk yeast-lactate liver medium (litmus milk containing 10% yeast-lactate-liver bouillon which was prepared by adding 5 g of yeast extract, 24 ml of sodium lactate and extract of fresh liver equal to 10 g of Bacto liver to 1000 ml of distilled water) which is a suitable medium for this purpose. Each culture of *Clostridium* was inoculated into the medium just described which was then incubated at 33 C for 10 days. This was found to be sufficient time for obtaining enough spores of each culture. At the end of the incubation period, the culture tubes were heated for 10 min at 85 C. Spores of each culture were counted using the direct microscopic technique (Breed's method) after staining the preparation with malachite green. The numbers ($\times 10^6$/ml) of spores found in different cultures were: *C. tyrobutyricum* NIZO, 1.00; *C. tyrobutyricum* 144, 0.79; *C. perfringens* 115, 0.47 and *C. sporogenes* T9, 7.00. *C. perfringens* did not sporulate well; an observation also reported by Sutton et al. (*10*).

Hydrogen peroxide

The hydrogen peroxide solution used was of medical quality and contained about 30% (w/v) H_2O_2. It was kept refrigerated and periodically was tested for strength. Determination (*2*) of H_2O_2 concentration was based on the reaction:



$$5\,H_2O_2 + 2\,MnO_4^- + 6\,H^+ \rightarrow 5\,O_2 + Mn^{++} + 8\,H_2O_2$$

Hydrogen peroxide solutions were freshly prepared for an experiment and added to tubes of sterilized litmus milk at concentrations of 0.01, 0.02, 0.04 and 0.06%.

RESULTS AND DISCUSSION

Occurrence of anaerobic sporeformers in Egyptian raw milk was studied by several investigators. El-Gendy (*4*) reported that such milk may contain between zero and 100 *Clostridium* spores per ml. The largest number (100 spores/ml) was detected only in six samples, whereas 75 of 100 samples were free of anaerobic sporeformers. Abo-El-naga (*1*) found that the number of anaerobic spores in some milk samples reached a mean of 16/ml.

In view of the numbers of spores found in milk, 50 and 100 spores of each culture used in this study were added

per milliliter of substrate to determine the effect of various concentrations of H_2O_2 on spore viability and growth.

Serial dilutions of each culture of *Clostridium* sp. were made in tubes of litmus milk to obtain 50 and 100 spores per ml. Freshly prepared hydrogen peroxide solution was added to the tubes in concentrations of 0.01, 0.02, 0.04 and 0.06%. Tubes were then heated at 50 C for 15 min to facilitate action of hydrogen peroxide and then sealed with vaspar. All tubes were incubated for 10 days at 33 C. Gas production and changes in appearance of litmus milk were used as criteria of growth of the microorganisms. Tables 1, 2, 3 and 4 give the results of these tests. Data in the tables are the result of four or more trials. The data show that 0.01% of H_2O_2 retarded the onset of growth and gas production by the *Clostridium* cultures for 6 to 8 days, depending on the species of *Clostridium*. Growth and gas formation by *C. tyrobutyricum* NIZO and *C. sporogenes* T9 at both concentrations of spores were inhibited by 0.02% of H_2O_2 during the entire incubation period. Slight growth of *C. tyrobutyricum* 144 and *C. perfringens* 115 appeared after 7 and 8 days of incubation, respectively, when these bacteria were treated with 0.02% of H_2O_2, whereas no gas appeared in these cultures, even after 10 days of incubation. The data also demonstrate that the smaller the initial number of spores, the more effective a given amount of H_2O_2 will be in inhibiting growth and gas

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TABLE 1. *Effect of H_2O_2 on growth and gas production by* Clostridium tyrobutyricum[a] *NIZO.*

Incubation time (days)	H_2O_2 (%) 0.00		0.01[c]	
	Growth	Gas	Growth	Gas
4[b]	+	–	–	–
5	+	–	–	–
6	+	+	–	–
7	+	+	–	–
8	+	+	–	–
9	+	+	+	+
10	+	+	+	+

[a]Similar results with inoculum of 50 or 100 spores/ml.
[b]No growth or gas production after 1, 2 and 3 days.
[c]No growth or gas production in the presence of 0.02, 0.4 or 0.06% H_2O_2.

TABLE 4. *Effect of H_2O_2 on growth and gas production by* Clostridium sporogenes[a] T9.

Incubation time (days)	H_2O_2 (%) 0.00		0.01[c]	
	Growth	Gas	Growth	Gas
3[b]	+	–	–	–
4	+	–	–	–
5	+	+	–	–
6	+	+	+	–
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+

[a]Similar results with inoculum of 50 or 100 spores/ml.
[b]No growth or gas production after 1 and 2 days.
[c]No growth or gas production in the presence of 0.02, 0.04 or 0.06% H_2O_2.

TABLE 2. *Effect of H_2O_2 on growth and gas production by* Clostridium tyrobutyricum *144.*

Incubation time (days)	H_2O_2 (%) 0.00		0.01		0.02[d]	
	Growth	Gas	Growth	Gas	Growth	Gas
3[a]	+[b] (+)[c]	– (–)	– (–)	– (–)	– (–)	– (–)
4	+ (+)	– (+)	– (–)	– (–)	– (–)	– (–)
5	+ (+)	– (+)	– (–)	– (–)	– (–)	– (–)
6	+ (+)	+ (+)	+ (+)	– (–)	– (–)	– (–)
7	+ (+)	+ (+)	+ (+)	sl (sl)[e]	– (sl)	– (–)
8	+ (+)	+ (+)	+ (+)	sl (sl)	– (sl)	– (–)
9	+ (+)	+ (+)	+ (+)	sl (sl)	sl (sl)	– (–)
10	+ (+)	+ (+)	+ (+)	sl (+)	sl (sl)	– (–)

[a]No growth or gas production after 1 and 2 days.
[b]Column of 50 spores/ml.
[c]Column of 100 spores/ml.
[d]No growth or gas production in the presence of 0.04 or 0.06% H_2O_2.
[e]sl = Slight.

TABLE 3. *Effect of H_2O_2 on growth and gas production by* Clostridium perfringens *115.*

Incubation time (days)	H_2O_2 (%) 0.00		0.01		0.02[d]	
	Growth	Gas	Growth	Gas	Growth	Gas
3[a]	+[b] (+)[c]	– (–)	– (–)	– (–)	– (–)	– (–)
4	+ (+)	+ (+)	– (–)	– (–)	– (–)	– (–)
5	+ (+)	+ (+)	– (–)	– (–)	– (–)	– (–)
6	+ (+)	+ (+)	+ (+)	– (–)	– (–)	– (–)
7	+ (+)	+ (+)	+ (+)	+ (+)	– (–)	– (–)
8	+ (+)	+ (+)	+ (+)	+ (+)	– (sl)[e]	– (–)
9	+ (+)	+ (+)	+ (+)	+ (+)	sl (sl)	– (–)
10	+ (+)	+ (+)	+ (+)	+ (+)	sl (+)	– (–)

[a]No growth or gas production after 1 and 2 days.
[b]Column of 50 spores/ml.
[c]Column of 100 spores/ml.
[d]No growth or gas production in the presence of 0.04 or 0.06% H_2O_2.
[e]sl = Slight.

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A Research Note

Use of Direct-Acid-Set Cottage Cheese Whey to Manufacture Sherbet

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ABSTRACT

Orange sherbet of 1% fat and 45 or 46% direct-acid-set cottage cheese whey were compared to a control sherbet containing no whey. The titratable acidity was higher in sherbets containing whey. An untrained taste panel found no difference between the three sherbets studied, describing them as "like moderately."

Sweet whey has been used in various applications in the food industry, but cottage cheese whey has limited usage, though it has been used in flavored drinks (*2*). Igoe et al. (*4*) used cottage cheese whey in ice cream mixes and found it unacceptable when more than 1% of the milk solids-not-fat were provided by acid whey. Hekmati and Bradley (*3*) used acid whey to make acceptable sherbet, the only criticism being a slight masking effect of the pineapple flavor.

Whey from cottage cheese made by the direct-acid-set (DAS) process does not have a fermented flavor and has a greater potential for use in foods than does whey from the culture-set method. The purpose of this study was to use whey from a DAS method for orange sherbet manufacture and determine its acceptability by a trained panel as well as an untrained panel.

MATERIALS AND METHODS

DAS cottage cheese whey was obtained from a commercial manufacturer, brought to the University of Tennessee Dairy Laboratory and used in formulation of orange sherbet. Each sherbet mix contained 1% fat, 4% serum solids, 0.4% stabilizer and 25.7% sweetener solids, part of which was from ice cream mix in sherbet mix II. The ice cream mix used contained 12% fat, 11% milk solids-not-fat, 14% sucrose and 1.6% corn syrup solids. The ingredients used in the control mix (no whey), and in the two experimental mixes are listed in Table 1. In the laboratory, 63 lb. (28.6 kg) of each mix were made and stored at 4 C for 20 h. The titratable acidity was determined by titrating with 0.1 N NaOH to the phenolphthalein end point and calculating the acidity as percentage of lactic acid. The total solids concentration was determined by drying a sample of the mix at 60 C in a vacuum oven at 250 torr.

The mix was frozen in a batch freezer designed to produce 10 gallons (37.9 liters) of product. The overrun was approximately 43%. The semi-solid product was withdrawn from the freezer and stored at -23 C until evaluted by a 20- to 25-member untrained panel. Two replications were conducted.

In preliminary laboratory work, an expert panel of six persons tasted orange sherbet made from Mix III. These persons were asked to state whether they could detect any defect in the sherbet due to use of whey in the formulation.

TABLE 1. *Composition of sherbet mixes.*

Ingredient	Mix I	Mix II	Mix III
	(%)		
Water	25.4	19.5	15.5
Whey	—	44.7	46.1
Milk	28.5	—	—
Skimmilk	17.2	—	—
Half and half	—	—	9.5
Ice cream mix	—	8.3	—
Sucrose	15.7	14.5	15.7
Dextrose	10.0	9.8	10.0
Citric acid	0.9	0.9	0.9
Lemon emulsion	0.2	0.2	0.2
Orange concentrate	1.7	1.7	1.7
Stabilizer	0.4	0.4	0.4

The untrained taste panel evaluation was conducted in the sensory laboratory with each panelist seated in an individual booth. Red fluorescent light was used to illuminate the samples. At each setting, panelists evaluated samples of sherbet for preference using a nine-point hedonic scale (*5*). A 1-inch diameter scoop was used to serve the samples at 1 to 2 C (*1*), and the order of presentation of samples was randomized. An average score for preference was calculated and the data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

The titratable acidity (Table 2) was higher in mixes with whey than in mixes without whey ($P < .01$). The pH values, however, were not different ($P > .05$). The total solids content, likewise, was not different among the mixes ($P > .05$).

The expert panel was unable to detect any defect in the whey-containing sherbet which could be attributable to

TABLE 2. *Chemical properties and flavor scores of sherbet.*

Property	MIX I	MIX II	MIX III
Titratable acidity (%)	0.61[a]	0.86[b]	0.85[b]
pH	4.0	3.5	3.5
Total solids (%)	31.3	31.7	32.0
Flavor score[1]	6.84	7.43	6.99

[1]9 = like extremely; 1 = dislike extremely.

Means bearing the same letter are not statistically different from each other. Those bearing different letters are statistically different $P < .05$.

the use of whey. The untrained taste panel detected no difference among the sherbets. There was no difference ($P > .05$) between overall mean preference scores of the sherbets. The flavor scores ranged from "like slightly" to "like moderately." The type of sensory evaluation used in this investigation was a screening test which indicated the desirability of cottage cheese whey in sherbet manufacture.

The results indicate that DAS cottage cheese whey may be used in the manufacture of sherbet to replace some water and milk solids without adverse consumer reaction. Though sherbet may be made using whey and no water, if such had been done in the present investigation, the lactose concentration of the experimental mixes would have been higher than that of the control mix. If 100% substitution is contemplated, the manufacturer might consider reducing the quantity of citric acid and using a fat source with low milk solids-not-fat concentration.

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formation. However, in all test cultures gas formation, the cause of the defect in hard cheese, was more readily suppressed by H_2O_2 than was growth. This was also reported by other investigators (*5,6,8*) when they studied the effect of nisin on growth and gas production by clostridia.

It is evident from our data that addition of 0.02% H_2O_2 was sufficient to inhibit clostridial growth and prevent gas production by the bacteria even when the number of spores present was comparatively large (50 or 100/ml). As already indicated, such a large number of clostridial spores rarely occurs in milk produced under ordinary conditions.

Hence our data suggest that hydrogen peroxide could be used to treat milk for cheesemaking to prevent the "late blowing defect." We also studied effects of hydrogen peroxide on lactic starter bacteria and use of hydrogen peroxide in manufacture of Swiss cheese. The results will be reported in subsequent papers.

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Estimate of Cases of Food- and Waterborne Illness in Canada and the United States

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ABSTRACT

Reports of outbreaks of food- and waterborne disease in Canada and the United States were searched for data on the number of cases of illness initially reported to health and food protection agencies. These were compared to the number of cases identified by thorough epidemiological investigations or to the number estimated. For a total of 51 outbreaks of bacterial, viral, and parasitic disease, the median ratio of the estimated cases to initially reported cases was 25:1. Based on this ratio and the systems of data transmission from the first level of reporting to national reporting agencies, the estimates of annual food- and waterborne disease cases for 1974 to 1975 were from 150,000 to 300,000 in Canada and 1,400,000 to 3,400,000 in the United States. For a total of 26 outbreaks of salmonellosis, the median ratio of estimated cases to initial human isolations of *Salmonella* was 29.5. Based on this ratio and reported isolations of *Salmonella* from 1969 to 1978, the estimates of all cases of human salmonellosis were 150,000 in Canada and 740,000 in the United States, annually.

It is generally accepted that reporting of outbreaks and cases of food- and waterborne diseases is inadequate at all levels of the health surveillance network. Frequently, persons with enteric ailments will not consult a physician or contact a health agency. Physicians often treat patients with enteric syndromes without taking specimens for laboratory confirmation. They seldom alert health agencies about possible outbreaks, largely because they may see or hear of only a few patients with gastroenteritis over a short period. Once alerted to alleged cases of food- and waterborne illness, health agencies often fail to (*a*) conduct thorough investigations that form hypotheses of food- and waterborne transmission, (*b*) prove such hypotheses or (*c*) submit reports of investigated outbreaks to surveillance agencies at state or provincial levels. In turn, not all outbreaks brought to the attention of these agencies are reported further. Data received by national surveillance agencies are, therefore, quite incomplete (*2,6,38*).

Reports of food- and sometimes waterborne disease outbreaks and cases are published periodically in the United States (*30*), Canada (*41*) and several other countries (*31,56,58,60*). At present, they seem to be more a reflection of the surveillance and reporting systems than of the actual incidence of food- and waterborne illness. For example, the eating habits and standards of hygiene are similar in the United States and Canada, yet the number of foodborne disease outbreaks reported for 1973-1975 per 100,000 population was 11 times higher in Canada than in the United States (*56*). The number of cases per 100,000 population was three times higher in Canada than in the U.S. These discrepancies indicate that a higher percentage of outbreaks, mainly those involving small numbers of cases, is reported in Canada than in the U.S.

Epidemiologists and food microbiologists, particularly in public health and regulatory agencies, are often asked to estimate the extent of foodborne illness in a given country, state or province. These estimates are usually requested to determine the cost of such illnesses and to justify expenditures for control programs, e.g. measures to reduce *Salmonella* contamination in poultry.

Several estimates of the annual occurrence of foodborne illness have been made. In 1960, Dauer and Davids (*34*) estimated 1,000,000 cases per annum in the United States. A year later (*33*) Dauer changed the estimate to 100,000 - 200,000. Buchbinder (*7*) estimated 500,000 to 1,000,000 cases per year. Todd and Pivnick (*57*) estimated the annul foodborne cases for Canada, which has a population of about 10% of the U.S., at 400,000.

In a survey for 1957 and 1958 covering 95 cities, 7 states, and 8 provinces with a combined population of 52 million, Fritz et al. (*39*) uncovered 417 outbreaks and 5,045 cases of foodborne disease in addition to the reported ones. An extrapolation indicated that about 1,000 outbreaks and 20,000 cases were investigated annually in the United States.

In 1969, a survey by Barker et al. (*5*) covering Washington State (population 3,000,000) revealed 69 investigated foodborne outbreaks and 1,135 cases. In contrast, only 371 food- and waterborne outbreaks and 28,563 cases were reported by the Center for Disease Control (CDC) for the total U.S. (population 200,000,000) (*30*). If the incidence of disease and the surveillance activity in Washington State were similar to those of the other states, about 4,600 outbreaks (76,000 cases) should have been investigated in the U.S. in 1969. The estimates of Fritz et al. (*39*) and Barker et al. (*5*) must be multiplied by factors of unknown magnitude to account for the still undetected foodborne outbreaks and cases and for

[1]*Health Protection Branch.*
[2]*Center for Disease Control.*

reported gastrointestinal illnesses that could not be conclusively linked to food.

An expert committee on *Salmonella* of the National Research Council (*4,32*) estimated that 2,000,000 cases of salmonellosis occurred annually in the U.S., compared to about 20,000 reported isolations of *Salmonella* from human stool specimens. This estimate was arrived at by collating the number of routine laboratory isolations of *Salmonella* arising from three large outbreaks of salmonellosis with the number of cases uncovered by intensive epidemiological and laboratory investigations. In one of these outbreaks which was waterborne (*53*), *Salmonella typhimurium* was isolated from 110 persons. Data from interviews in a sample population of the community at risk revealed 16,000 cases. In an outbreak from barbecued turkeys served at a community celebration (*4,24*), only five isolations of *Salmonella* were reported to the national surveillance system. Subsequent interviews of 1,059 participants revealed that 287 of these (27.1%) had suffered diarrheal illness. By applying this attack rate to the 7,000 persons who had attended the event, 1,900 cases were estimated. In the third outbreak (*3*), caused by an egg-containing frozen kosher dessert item that was served at several catered parties in different states, 34 isolations were made. However, a thorough investigation estimated about 1,800 cases.

Although the means by which the Committee (*32*) derived its estimate are basically valid, the estimate is weakened by a degree of conjecture and the use of data from only a small number of exceptionally large outbreaks. Until more reliable surveillance data become available, actual numbers of outbreaks and cases of food- and waterborne illness cannot be derived from reported numbers without considerable conjecture. The aim of the present study was to review a large number of well documented outbreaks to form a base for estimating the incidence of food- and waterborne illness in Canada and the United States.

METHODS

Published and unpublished reports of food- and waterborne outbreaks were reviewed. Data were sought to relate cases of illness reported through normal channels to cases subsequently uncovered by thorough epidemiological investigations. Only a very small percentage of these contained all the essential information, but missing data could often be obtained from unpublished documents or through personal communication.

Definition of terms used in tables: (*a*) *Initially reported ill* - The number of persons initially reported to the local health authority, or known at the time an investigating team arrived on the scene. (*b*) *Initial human isolations* - Isolations of pathogens from specimens that were collected before an intensive investigation. (*b*) *Ill identified* - Number of cases identified by clinical or laboratory means and reported to the federal surveillance agency or in a published report. (*d*) *Number at risk* - Number of persons who attended the common meal or event, or ingested the contaminated food or water. (*e*) *Attack rate* - Quotient of the number ill identified divided by the number of persons at risk interviewed, times 100. (*f*) *Estimated ill* - Number at risk times attack rate. (*g*) *R* - Ratio of the estimated ill to the initially reported ill. (*h*) *Ri* - Ratio of the estimated ill to the initial human isolations.

Some of the data presented are explained below: *Outbreaks of salmonellosis* (*Table 1*). For outbreaks 1, 5 and 15, definite numbers of "initially reported ill" could not be obtained. The corresponding ratios (R) of estimated ill to initially reported ill, therefore, lack precision.

Human isolations of *Salmonella* that were obviously made as a result of the epidemiological investigations were not included in the table. However, it was sometimes difficult to distinguish initial isolations and isolations made as a result of the investigation. This may have raised some of the figures under "initial human isolations," and thus lowered the ratio of estimated ill/initial human isolations (Ri).

The "ill identified" figures are approximate only. This is of little consequence, however, because the data were not used for any of the estimates.

The "number at risk" were known in about half the outbreaks and represent broad estimates in the other half, particularly the large ones, for example 1, 5, 8 and 12. Nearly all of the attack rates could be recalculated from survey data. Attack rates were quoted from reports only for outbreaks 1, 5, 15, 20, 23 and 24. The "esimated ill" figures were based on attack rates, with the exception of outbreaks 2 and 7. In these outbreaks, previously unreported cases were uncovered by searches of hospital records, a media alert that linked illnesses to a specific food, and a community survey. Cases uncovered by these means are likely to remain incomplete.

The ratios R and Ri (Table 1) with + or ± signs were calculated from somewhat incomplete data. With the possible exception of outbreak 2, these errors seemed to be small relative to the nature of this study. The + and ± signs were, therefore, not considered in calculating the median ratios.

Outbreaks 6 and 7 were associated with the same food outlet and investigated as a result of a single reported case. Similarly, outbreak 18 was investigated after isolation of *Salmonella* from a Rhode Island resident who had recently returned from a Caribbean cruise. The survey was limited to Rhode Island residents who had taken the same cruise but prompted the investigation of outbreak 19 involving 7 subsequent cruises. Outbreaks 9 and 10 are essentially sub-incidents of outbreak 8.

Bacterial outbreaks other than salmonellosis (*Table 2*). Outbreak 3 was discovered only because it was mentioned privately in the presence of a health official; none of the cases had been reported. For outbreak 4, only a minimum number of "initially reported ill" were identified; the actual number is likely somewhat higher.

Outbreaks of hepatitis A and trichinosis (*Tables 4 and 5*). In contrast to the outbreaks listed in Tables 1-3, the numbers at risk and of people ill could not be established. Therefore, the ratios of ill identified/initially reported ill, which are somewhat lower than the comparable ratios (R) of estimated ill/initially reported ill, are given.

RESULTS

Data from 26 outbreaks of salmonellosis are listed in Table 1. The ratios of estimated ill/initially reported ill (R) varied from 3 to 526, with a median ratio of 29.5. Ratio Ri varied from 3 to 379, also with a median of 29.5.

The spread of R was similar for outbreaks of other bacterial illnesses (Table 2) and of giardiasis (Table 3). The medians were 25 and 22, respectively.

Tables 4 and 5 show the ratios of ill identified/initially reported ill for cases of hepatitis A and trichinosis. The median ratio was 9 for both diseases.

A median value of R = 25 was obtained when the data of Tables 1-5 were considered together. The ratios in Tables 4 and 5 are not strictly comparable to R because not all the ill persons are likely to be identified in an outbreak. However, the median calculated from Tables 1-3 alone was not significantly different (R = 25.5).

Table 6 shows the application of R and Ri to reported cases of food- and waterborne illnesses and to human *Salmonella* isolations, respectively. If available, surveil-

TABLE 1. *Under-reported food- and waterborne cases of salmonellosis.*[a]

Outbreak	Vehicle	Initially reported ill	Initial human isolations	Ill identified	No. at risk	Attack rate (%)	Estimated ill	Estimated ill/ initially reported ill (R)	Estimated ill/ initial human isolation (Ri)	Main basis for estimate	Reference
1	Turkey roll	30-44	10	44	1,400	18	250	6-8	25	Questionnaire, attack rate	*19*
2	Custard-filled doughnuts	5	5	100 +	UNK	UNK	100 +	20 +	20 +	Media, Hospital records	*20*
3	Banana cream pie	88	12	135	700	43.7	306	3	25	Questionnaire, attack rate	*21*
4	Delicatessen foods	3	3	18	23	78	18	6	6	Interviews	*22*
5	Turkey	10-61	29	65	1,000	85	850	14-85	29	Questionnaire, attack rate	*23*
6	Barbecued chicken	1	1	25	56	49	27	27	27	Community survey, attack rate	CDC surveillance data, 1966
7	"	1	1	82	UNK	UNK	82 +	82 +	82 +	Community survey	"
8	Meringue cream pies	27	27	244	3,000-6,000	50 ±	2,200 ±	80 ±	80 ±	Attack rate, contam. lot size	*1*
9	Chocolate meringue pie	1	1	214	719	34.1	245	245	245	Questionnaire, attack rate	*1*
10	Banana meringue pie	2	2	30	180	46.2	83	42	42	Telephone survey, attack rate	*1*
11	Lemon meringue pie	64	12	129	719	47.4	341	5	28	Questionnaire, attack rate	*1*
12	Turkey	200	5	287	7,000	27.1	1,897	9	379	Survey, media, attack rate	*24* CDC surveil-data, 1968
13	Delicatessen foods	198	63	240	4,000	45.9	1,836	9	29	Media, interviews, attack rate	*46*
14	Turkey salad	1	26	122	1,900	27.7	526	526	20	Telephone survey, questionnaire, attack rate	CDC surveil-data, 1969
15	Kosher dessert with eggs	100-196	34	196	3,450 +	52	1,794	9-18	53 +	Survey, attack rate	*3*
16	Water	197	110	1,035	133,219 +	11.8	16,000	81	145	House-to-house survey, attack rate	*53*
17	Probably food	118	85	318	1,106	30.4	336	3	4	Questionnaire, attack rate	*8* CDC surveillance data, 1974
18	Probably food	1	1	16	500 ±	35.6	178	178	178	Survey, attack rate	*25*
19	" "	1	1	302	3,884	7.8	302	302	302	" "	*25*
20	Cold beef	8	47	376	1,000	60	600	75	13	Survey, attack rate	*40* Groisbois, pers. comm.
21	Cheddar cheese	28	149	339	31,840	12.9	4,107	143	28	Survey, attack rate	*12* CDC surveillance data
22	Roast beef	1	1	19	62	51.4	32	32	32	Survey, attack rate	*10*
23	Cold ham and/or turkey	5	5	82	124	79	98	20	20	Survey, attack rate	*44* MacIntyre, pers. comm.
24	Turkey	5	5	65	111	67	74	15	15	Survey, attack rate	*44* MacIntyre, pers. comm.
25	Banquet dinner	4	2	89	290	35.4	103	26	52	Questionnaire, attack rate	*45*
26	Probably turkey	2	13	379	1,160	33.6	390	195	30	Questionnaire, attack rate	*18*

[a]See Methods section for explanation.

TABLE 2. *Under-reported food- and waterborne bacterial cases other than salmonellosis.*[a]

Outbreak	Disease	Vehicle	Initially Reported ill	Ill identified	No. at risk	Attack rate (%)	Estimated ill	Estimated ill/ initially reported ill (R)	Main basis for estimate	Reference
1	Group A streptococcal infection	Egg salad	< 60	60	800-900	69.8	600 ±	10 +	Questionnaire, attack rate	*37*
2	*C. perfringens* gastroenteritis	Lamb stew	47	110	744	42.2	314	7	Questionnaire, attack rate	*49*
3	*C. perfringens* gastroenteritis	Beef in gravy	0	29	150	67.4	101	101 +	Telephone survey, attack rate	*50*
4	Gastroenteritis	Water	18 +	281	15,000	16.7	2,500	139 -	Survey, attack rate	*52*
5	Shigellosis	Unknown food	6	112	165	89.6	148	25	Questionnaire, attack rate	*26*
6	Shigellosis	Probably water	3	170	650	56.7	369	123	Interviews, attack rate	*42*
7	Yersiniosis	Water	30	129	1,550	41	750	25	Questionnaire, attack rate	*36*
8	*V. parahaemolyticus* gastroenteritis	Seafood salad	36	98	1,059	9.5	101	3	Questionnaire, attack rate	*16*
9	*Campylobacter* gastroenteritis	Water	200 ±	UNK	10,000	14.4-23.0	2,000 ±	10	Household survey	*17*

[a]See Methods section for explanations.

lance data over periods of up to 10 years were used to calculate the average numbers of cases or isolations. Since Canadian food- and waterborne data have been reported for 1974 and 1975 only, the U.S. cases were presented separately for this period. The data on *Salmonella* isolations refer to all cases of salmonellosis, not to food- and waterborne cases alone.

DISCUSSION

If it is valid to estimate cases of salmonellosis on the basis of Ri, as done by the Committee on Salmonella, National Research Council (*4,32*), valid estimates of food- and waterborne cases, including salmonellosis, may also be made by applying ratio R to the number of reported cases. Unfortunately, the number of outbreaks for which all the information relevant to R could be obtained is still small. The value of this study is also somewhat diminished by the fact that not a single staphylococcal outbreak was among them. Chemical poisonings were also not included, but these comprise only a small percentage of reported cases. Because of the difficulty in separating initially reported cases from reported cases that were discovered during the investigation, some of the "initially reported ill" figures are vague. In contrast, the numbers of persons at risk and the attack rates are relatively accurate.

Application of R to individual small outbreaks tends to over-estimate the number of ill involved. On the other hand, the probability that no cases of illness will be reported increases as the number of cases involved in an outbreak decreases. The over-estimation of cases for small reported outbreaks, therefore, seems to be off-set by the cases of entirely unreported outbreaks.

The factor likely to affect R the most is the type and location of an outbreak. For example, R is expected to decrease with the severity of the illness, i.e., botulism outbreaks involving more than one person are unlikely to remain undetected. R is also expected to be low if the victims remain in close proximity, i.e., on board ships and in hospitals, school residences and prisons, or where medical service is readily available. Outbreak 8 (Table 2) occurred on a cruise ship; if the incriminated salad had been served in a restaurant, the number of reported cases would probably have been much smaller.

Despite the above qualifications, the results of Tables 1-5 suggest that about one of 25 cases of foodborne or waterborne illness would be initially reported. The median ratio of estimated ill/human isolations (Ri) was 29.5 and thus considerably lower than the value of 100 estimated by the Committee on Salmonella (*32*). The latter value, however, was based solely on data of outbreaks 12, 15 and 16 (Table 1), for which Ri ratios were exceptionally high.

It must be stressed that the cases of food- and waterborne illness reported at the federal level (Table 6) are lower than the case numbers reported to local and state/provincial agencies, depending on the system of data transmission between these levels. Differences in the

transmission of data between the U.S. and Canada may explain the differences in reported outbreaks and cases per 100,000 population between these two countries.

It is estimated (Todd, personal communication) that, through close personal liaison between the agencies involved, at least 50% of the reported and verified cases of food poisoning in Canada are presently transmitted by municipal and provincial agencies to the Health Protection Branch and collated in the annual summaries. To correct for a loss of 0-50% of cases in the data transmission in Canada, the product No. × R (Table 6) must be multiplied by a factor of 1-2.

Due to a ten-fold greater population, and transmission of data on a less personal basis, this factor appears to be substantially larger for the United States. However, any attempt to quantify the factor can only be tentative at this time. The survey of Fritz et al. (*39*) suggested that about 50% of the foodborne cases investigated in the

TABLE 3. *Under-reported cases of giardiasis.*[a]

Outbreak	Vehicle	Initially reported ill	Ill identified	No. at risk	Attack rate (%)	Estimated ill	Estimated ill/ initially reported ill (R)	Main basis for estimate	Reference
1	Water	3	70	399	22.0	88	29	Questionnaire, attack rate	*29*
2	Water	165	359	50,148	10.6	5,316	32	Household survey, attack rate	*55* Lyman, pers. comm.
3	Water	4	72	179	40.7	73	18	Questionnaire, attack rate	*9*
4	Probably water	1	86	199	58	115	115	Questionnaire, attack rate	*43* Mathias, pers. comm.
5	Water	3	27	65	41.5	27	9	Questionnaire	*13*
6	Water	32	128	6,000	3.8	228	7	Questionnaire, attack rate	*14*
7	Water	90	205	15,000	13	1,950	22	Questionnaire, attack rate	*14*

[a]See Methods section for explanations.

TABLE 4. *Under-reported foodborne cases of hepatitis A.*[a]

Outbreak	Vehicle	Initially reported ill	Ill reported	Ill identified/ initially reported ill	Main basis for estimate	Reference
1	Raw clams	6	459	76	Case finding, survey	*35*
2	Raw clams	4	123	31	Case finding, survey	*54*
3	Cold sandwiches	12	107	9	Case finding, survey	*48*
4	Restaurant foods	22	62	3	Case finding, questionnaire	*47*
5	Submarine sandwiches	9	18	2	Case finding, telephone interviews	*15*

[a]See Methods section for explanations.

TABLE 5. *Under-reported food-borne cases of trichinosis.*[a]

Outbreak	Vehicle	Initially reported ill	Ill identified	Ill identified/ initially reported ill	Main basis for estimate	Reference
1	Ham salad cross-contam. with raw pork	3	77	26	Survey of hospital records	*51*
2	Summer sausage	15	44	3	Survey of clinical records and food histories	*59*
3	Beef cross-contam. with pork	5	13	3	Survey of ship passengers	*27*
4	Pork-venison sausage	5	73	15	Survey	*11,28*

[a]See Methods section for explanations.

TABLE 6. *Application of R and Ri to cases of food- and waterborne illness, and to* Salmonella *isolations from humans.*[a,b]

Reported cases or isolations	Country	Years	No. reported annually: Range × 1000	No. reported annually: Average	No. × R[c] or No. × Ri[d]
Food- and waterborne cases	Canada[e]	1974-'75	4.6-7.6	6,100	150,000
	U.S.	1974-'75	24-29	27,000	680,000
		1967-'76	14-29	21,000	530,000
Human isolations of *Salmonella*	Canada	1969-'78	4.3-8.5	5,200	150,000
	U.S.	1969-'78	21-29	25,000	740,000

[a]Surveillance data of Center for Disease Control, Atlanta, and Health Protection Branch, Ottawa.
[b]Figures to the first two significant numbers.
[c]R = 25, for food- and waterborne cases.
[d]Ri = 29.5, for *Salmonella* isolations.
[e]Canadian food- and waterborne cases reported only for 1974 and 1975.

U.S. in 1957 and 1958 were reported to the federal level. From the survey of Barker et al. (*5*) 76,000 foodborne cases were extrapolated to have been investigated in the U.S. during 1968, while CDC reported a total of 28,563 cases of food- and waterborne illness for the same year (*30*). For the years 1971 to 1976, when food- and water-related cases were tabulated separately, the mean number of foodborne cases was 14,445 annually (*30*). Accepting the extrapolation data of Barker et al. for comparison with cases reported by CDC and assuming the same degree of transmission for foodborne and waterborne cases, we arrive at an efficiency rate of 20% for transmission of investigated cases to the federal level in the U.S. The loss of 50-80% of cases may be corrected by multiplying No. × R (Table 6) by a factor of 2-5.

Application of the correction factors to the data in Table 6 would indicate 150,000 to 300,000 food- and waterborne cases annually in Canada for 1974 to '75. Our estimates for the U.S. are 1,400,000 to 3,400,000 for the same period and 1,100,000 to 2,600,000 for the period of 1967-'76.

The above type of correction factor is not applicable to No. × Ri (Table 6) because the *Salmonella* surveillance systems both in Canada and the U.S. assure that essentially all verified isolations of *Salmonella* are transmitted to the federal agencies. The data in Table 6, therefore, indicate, for a 10-year period, 150,000 and 740,000 cases of human salmonellosis annually in Canada and the U.S., respectively.

The means by which we arrived at these figures are certainly open to criticism, but we hope that this work, despite its many shortcomings, may stimulate investigators of food- and waterborne outbreaks to record data that may help to update and revise these estimates.

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Yield and Curd Characteristics of Cottage Cheese Made by the Culture and Direct-Acid-Set Methods[1]

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ABSTRACT

We compared yield and curd characteristics of cottage cheese made by the short-set culture and direct-acid-set methods using three skim milk protein concentrations, 3.1, 3.5 and 3.9 ± .1%. For each method of manufacture, approximately 380 kg of the same skim milk were set per experimental vat. Representative samples of whey, wash water and curd were analyzed and the total quantities of each were measured. Solids and protein recovered in whey, wash water and curd were related to yields for each method. The same curd samples also were used to measure curd size distribution, curd firmness and dressing retention. Analysis of variance showed highly significant differences in curd yield between the two methods when the three protein concentrations were considered. There was approximately 5% more yield when cottage cheese was made from skim milk containing 3.1 or 3.5% protein, using the direct-acid-set method. This yield advantage was less than 1% when the skim milk contained 3.9% protein. Curd firmness did not differ significantly between methods ($p < .01$). Curd from the 3.1% protein-skim milk, however, was firmer ($p < .01$) than that from either 3.5 or 3.9% protein-skim milk. Direct-acid-set curd was more uniform in size than that of the short-set culture curd. It retained dressing better only when made from 3.1% protein skim milk, and when 1.25 × the normal amount of dressing was used.

Mabbitt et al. in 1955 (*15*) reviewed early attempts to make cheese by substituting acidulants for bacterial starters. They also used lactic or hydrochloric acid or gluconic acid lactone to manufacture Cheddar or Cheshire-type cheese. Deane and Hammond in 1960 (*5*) used D-glucono-delta-lactone and mesolactides in manufacturing cottage cheese. These compounds hydrolyze slowly in solution to produce acids. When added to milk, they induce a characteristic coagulum while the milk remains quiescent. Hammond and Deane patented that process in 1961 (*13*). In 1963, Ernstrom patented a process for cottage cheese using hydrochloric acid in place of the more expensive gluconolactone (*8*). Then in 1971 Corbin (*2*) developed and patented a batch procedure using phosphoric acid as the initial and partial acidulating agent and D-glucono-delta-lactone for final milk acidification. This batch process was approved as another method of manufacture in the Standards of Identity for Cottage Cheese Dry Curd (*9*).

Vitex-American Laboratories introduced an in-line acidification system similar to Corbin's patented method (Vitex 750-850 system™)[1], based on a modification of the Hammond-Deane patent (*10,20*). This process, which involves continuously metering Vitex 750™ into the cold milk instead of adding the acidulant in batch, has been accepted commercially by some plants. Gerson (*11*) predicted that 8-10% of cottage cheese made in the U.S. would be made by the direct-set method by the end of 1977, and later estimated (*12*) that about 17% of the market of cottage cheese now manufactured in the U.S.is made by the direct-set method.

Replacing the starter culture method with a direct-set method eliminates the possible problems of bacteriophage, antibiotics and slow cultures, and at the same time reduces the manufacturing time by almost half. Such a process, however, must yield a quality product and be economical.

White and Ray (*22*) reported lower yields for cottage cheese made by the direct acidification method than by other methods when yields were expressed as curd per kilograms of solids, disregarding moisture content of the curd. Lower yield for the direct acidification method, however, reflected more total solids in the curd. Although the yields were lower, solids recovered were similar for the direct acidification and continuous fermentation methods and as high or higher than from other methods when curd solids were adjusted to 20%. High curd solids reported by White and Ray do not appear to be characteristic of the direct-acid-set method and may have reflected overcooking.

Recently Satterness et al. (*19*) compared the direct-set method and the culture method, using an experimental design similar to the one reported in this paper. It differed, however, in the following ways: we standardized the protein content in the skim milk, they did not; we used in-line acidification for initial acidification of cheese milk, whereas they used a batch system. In addition, we compared a number of cottage-cheese curd properties from the two methods.

Satterness et al. (*19*) compared the direct-set and culture methods with three types of milk: fresh, fortified and reconstituted skim milk. They found no significant ($p < .05$) difference in yields but the direct-set method yielded significantly less curd fines.

We compared yields and properties of cottage cheese made by the culture and the direct acidification methods at each of three protein concentrations in skim milk.

[1]*Contribution No. 78-410-J, Department of Animal Sciences and Industry and Statistics, Kansas Agricultural Experiment Station, Kansas State University, Manhattan 66506.*

MATERIALS AND METHODS

Experimental design

Raw skim milk with 3.1, 3.5, or 3.9 ± .1% protein was pasteurized, divided into two lots and manufactured into cottage cheese by the culture and direct-acid-set methods on the same day. Nine replicate pairs of each skim milk protein concentration were made into cottage cheese over 2 to 3 months and the two methods compared for yields and curd characteristics. The six treatment combinations were analyzed as a split-plot design with error (*a*) mean squares equal to replication within protein and error (*b*) mean squares estimated from residual variation.

Manufacturing methods

Milk for the 3.1% protein concentration was collected from the Kansas State University dairy herd. The 3.5 and 3.9 ± .1% protein milks were obtained by blending milks from the Kansas State University herd and a local Guernsey herd. Milk was separated and pasteurized (72.5 C/16 sec) in the Kansas State University dairy on the day received, held at 2 to 5 C, and made into cottage cheese the next day. The two cheese vats used were 378.5-liter (100 gal) and 757-liter (200 gal) capacity, and were alternated between the two methods throughout the study. Approximately 380 kg of skim milk was set in each vat.

Culture cottage cheese was made by the short-set method using 5% Hansen's Culture #56. Vitex Cottage Cheese Coagulator was added in the amount of 5.8 ml/100 kg of skim milk, and the curd was cut with 9.5-mm curd knives according to a positive AC test at pH about 4.7. Cooking procedures were essentially as described by Emmons and Tuckey (*7*). Final cooking temperatures varied from 47-57 C. Tap water was added to cool the curd to 27 C for the first wash and acidified (pH 4.5) chlorinated (10 ppm) ice water (4.5 C) was added to chill the curd to 7 C. In each instance whey or wash water was collected, measured and while thoroughly mixing, sampled. After the second wash the curd was drained until the drain rate reached 1 ml per min per 3.78 liters of skim milk set. After thoroughly mixing, samples of curd were collected for analysis and testing.

Direct-acid-set cottage cheese was made according to Vitex/American procedures (*20*). We added a prepared food-grade acid mixture, Vitex 750™, thru an in-line mixer directly to 2 to 5 C pasteurized skim milk to adjust the pH to 5.1 ± .15. The milk was heated to 32 C and based on the pH and weight of milk a measured amount of D-glucono-delta-lactone (Vitex 850™) and the Vitex coagulator (13 ml/100 kg skim milk) were added. The acidified milk was mixed thoroughly and maintained at 32 C for 1 h. After cutting the curd, approximately 89 ml of Vitex 750 per 378.5 liters of skim milk was added to adjust the pH of the whey to 4.4-4.5. Cooking, draining and sampling were the same as with the culture method.

Analytical procedures

Skim milk was measured by volume in the vats with a dip stick and curd was weighed on the creamery scales. Solids were determined gravimetrically (*5*); both total protein and casein were determined by AOAC methods (*1*). Curd size was measured by the method of Kosikowski (*14*), and curd fines by the method of Raab et al. (*18*). Curd firmness was determined by the method of deMan (*16*), using a Kramer Shear Press and expressed as shear value in kg/100 g of curd and dressing retention by a modification of the method of Emmons and Price (*6*). This modification involved first adjusting curd to 20% solids by draining a measured weight of whey from known amounts of curd that had previously been analyzed for total solids and dressing 100 g curd with 44, 55, 66 or 88 g of 14% fat-dressing containing sufficient salt to give 1% in the final product. The salted dressing and curd were mixed and stored in a closed carton at 5 C for 24 h. The mixture then was remixed and transferred to a circular 8-mesh screen placed horizontally in a 15-cm funnel to hold the cheese. Sheets of aluminum foil were used to cover the curd to minimize drying while dressing drained into tared 100 ml cylinders. After 30 min, the cylinders were reweighed and amounts of dressing retained calculated by difference and expressed as percentage of added dressing.

RESULTS AND DISCUSSION

Curd yields

The direct-acid-set method of making cottage cheese produced higher average yields ($p < .001$) than the culture method when expressed as kg of curd per 100 kg of skim milk, per kg of protein, per kg of casein, or per kg of total solids (see Table 1).

Increasing the protein concentration in the skim milk increased yields ($p < .001$) when based on 100 kg of skim milk and means of the two methods were combined. When comparing yields as kg of curd per kg of protein or kg of total solids, yield means from the 3.5 and 3.9% protein skim milk did not differ; however, the yield from 3.1% protein skim milk was lower ($p < .01$), (Table 1).

TABLE 1. *Analysis of variance and mean for curd and solids recovery.*

		Mean square							
		Curd recovered per				Solids recovered in			
Source of variation	df	100 kg of skim milk	kg of protein	kg of casein	kg of solids	Whey	1st wash	2nd wash	Curd
Protein	2	86.36***	.268**	.014	.3736***	231.6***	.728	1.816	151.701***
Rep/prot.	24	.68	.045	.070	.0061	17.5	12.674	2.282	2.569
Method	1	2.58***	.210***	.465***	.0315***	312.2***	159.412***	18.680***	11.612***
Method × protein	2	.52	.037	.110*	.0064*	39.5	14.332	.611	2.442
Residual	24	.16	.015	.021	.0014	13.4	6.117	1.394	.807
		Means (%)							
Method									
Culture		16.41	4.68	Because interaction is		45.17	9.35	4.73	35.34
Direct-set		16.84	4.81	significant, see Table 4 for		49.98	12.79	5.91	36.27
		(.225)[1]	(.070)	the protein, protein ×		(2.05)	(1.39)	(.663)	(.505)
Protein (%)				method means					
3.1		14.36	4.60			51.68	11.03[a]	5.62[a]	32.70
3.5		16.78	4.81[a(2)]			46.01	11.28[a]	4.99[a]	36.27
3.9		18.73	4.82[a]			45.03[a]	10.89[a]	5.35[a]	38.45
		(.575)	(.146)			(2.88)	(2.45)	(1.05)	(1.10)

*Significant at 5%
**Significant at 1%
***Significant at 0.1%
[1]$LSD_{.05}$ are bracketed.
[2]Means not significantly different at the 5% level are joined by a common letter.

Yields did not differ at the three protein concentrations when based on kg curd/kg casein; this merely reflects the correlation between casein and yield of cottage cheese. Individual average yields for both methods and each concentration of protein are presented in Table 4.

Figure 1 illustrates the effect of method and skim milk-protein concentration on yield. The nearly parallel lines of the two methods between 3.1 and 3.5% protein represent a similar rate of increase in curd yield for the two methods from increased protein concentration in the skim milk. The direct-set method produced approximately a 5% greater yield than the culture method for normal mixed herd milk (3.1 or 3.5% protein). We cannot explain the convergence of the lines (Fig. 1) representing a loss in advantage of yield in the high-protein (3.9%) milk. From a practical point of view, however, this is of little consequence with today's milk supply.

In comparing yields by the two methods and at protein concentrations of 3.1 and 3.5%, differences were more distinct (Table 2) than when all three protein concentrations were considered. The direct-set method produced higher yields ($p < .001$) than the culture method for all four methods of expressing yields. Those from 3.5% protein-skim milk were higher ($p < .001$) than from 3.1% protein except when expressed as kg curd/kg casein. The method × protein interaction that occurred with the three protein concentrations (Table 1) disappeared when only the two protein concentrations (Table 2) were considered.

Satterness et al. (*19*), in comparing cottage cheese yields obtained using the culture and direct-set methods and fresh skim milk containing an average 3.04% protein, reported no difference ($p < .05$) due to method. In our study, the yield differences ($p < .001$) between these two methods from skim milk containing 3.1 ± .1% protein reflected less variability than did data of Satterness et al. among replicates.

Factors contributing to uniformity among our

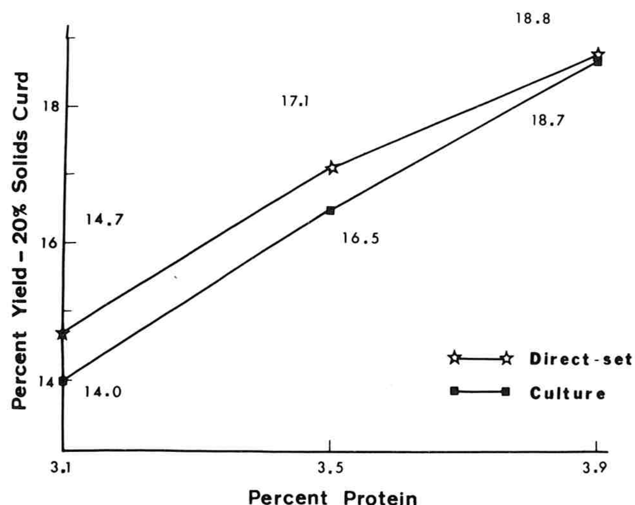

Figure 1. *Average yields of cottage cheese by culture and direct-acid methods from skim milks containing three protein concentrations.*

TABLE 2. *Analysis of variance of yields between two methods of making cottage cheese at each of two protein concentrations.*

Source variation	d.f.	Mean squares of curd recovered per 100 kg of skim milk	kg of protein	kg of casein	kg of solids
		Mean square kg			
Protein	1	52.80***	.386***	.027	.273***
Rep/prot.	16	.330	.018	.043	.002
Methods	1	3.61***	.283***	.672***	.044***
Methods × Protein	1	.00	.000	.012	.000
Residual	16	.100	.014	.017	.001
		Means[1]			
Methods					
Culture		15.25	4.62	6.19	1.69
Direct-set		15.89	4.79	6.46	1.76
		(.225)[2]	(.084)	(096)	(.021)
Protein					
3.1%		14.36	4.60	6.30[a]	1.64
3.5%		16.78	4.81	6.35[a]	1.81
		(.405)	(.091)	(.146)	(.033)

***Significant at 0.1%

[1]Means not significantly different at the % level are joined by a common letter.

[2]$LSD_{.05}$ are bracketed.

replicates were protein standardization and in-line acidification of the skim milk. Because protein concentrations in skim milk contribute to cottage cheese yields, we standardized milk into three groups: 3.1, 3.5, and 3.9 ± .1% protein. In-line acidification, in which the initial acidulating agent is metered into the milk at a constant rate, is an improvement over the batch addition of acid and probably reduces variability. Certainly the relatively large volume of milk we set, 380 kg, tended to reduce variations due to small errors in measurements. These factors contributed to precision and the small variability among replicates.

The yield means by the two methods in both of these studies were remarkably similar. Our yields expressed as kg of curd/100 kg of skim milk were 14.04 and 14.68 for culture and direct-set, respectively, and theirs (*19*) were 15.25 and 15.94. Our difference between methods was .64, and theirs (*19*) was .69--both in favor of the direct-set method.

The high fat content in skim milk used by Satterness et al. (*19*) resulted in an excess fat recovered in the curd. This contributed to 1 to 2% higher yields for both methods from fresh or fortified skim milk. Fat in the cheese curd was 1.68 to 2.46%; whereas, the fat content of uncreamed curd would be less than 0.4% (*21*) from efficiently skimmed cheese milk.

Total solids recovery

A total solids and protein accountability study was designed to help explain difference in yields. The acids added, Vitex 750™, Vitex 850, and GDL contributed to the solids in the whey and wash waters but probably only to a small degree in the curd. Table 1 presents these solids recovery data without including GDL as part of the total milk solids used. Partial accountability was made for the Vitex 750™ because the volume measurement used in the vat included the liquid acid added. The

added acidulants are reflected in our results for solids recovered in direct-set whey and wash waters (Table 1). Milk solids both with and without GDL were used to calculate total solids distributed in whey, wash waters and curd, as shown in Table 4a. When GDL was considered as part of the milk solids entering the vat, total solids in the whey, wash waters and curd were lower.

Table 1 shows that more milk solids were recovered in the curd by the direct-acid method than by the culture method ($p < .001$). This probably reflected less protein lost in the whey by the direct-acid-set method (Table 3). Differences in solids recovered in the curd were greater from 3.1 and 3.5% protein skim milk than from 3.9% (see curd less GDL, Table 4a).

Increasing the protein in the skim milk (3.1, 3.5, and 3.9%) decreased solids lost in the whey and increased solids recovered in the curd when means for the two methods were combined ($p < .001$). Total solids lost in the first and second wash did not differ ($p < .01$) at each protein concentration.

Protein recovery

Results of protein accountability are presented in Table 3. Protein recoveries distributed among wash water and curd by the two methods and for the three protein concentrations did not differ significantly ($p < .01$). More protein, however, was lost in the whey by the culture than the direct-set method ($p < .05$). Increasing protein in the skim milk (3.1 and 3.5%) reduced protein lost in the whey ($p < .01$) when means for the two methods were combined. Protein lost in the whey, however, did not differ when the protein level was increased to 3.9%.

Curd size distribution

Curd size distribution for each protein concentration and for the two methods is shown in Table 4b, and results of the statistical analysis for the large and small curd particles (eg. those retained on 12.7 mm (½ inch) and 1.4 mm (1/18 inch) sieves, combined) are presented in Table 3. Particle sizes are important because they indicate problems with cheese manufacture. Small curd particles, those deposited on a 1.4-mm sieve, are called "grit." A high grit value is undesirable in cottage cheese because it indicates curd shattering and poor yields (*3*). Particles retained on a 12.7-mm sieve indicate matting, more common to the culture than the direct-set method. A combination of curd particle sizes distributed on 12.7-mm and 1.4-mm (1/2 + 1/18 inch) sieves was chosen because matting may be associated with shattering (resulting from excessive agitation necessary to break lumpy curd). Table 3 shows more large and small curds from the culture (8.09) than from the direct-acid method (5.85) ($p < .05$). Because skim milk for both methods was identical, and the personnel involved in making the cheese were the same, the method must account for difference in the curd particle size. Lower mean values for the combined particle sizes for the 12.7-mm and 1.4-mm sieves for the direct-acid method ($p < .01$) indicate more uniform curd size. Increasing the percent protein in the skim milk (3.1, 3.5, 3.9%) did not affect the combined curd particle size distribution significantly when the means for the two methods were combined.

Curd fines, firmness and dressing retention

We also measured but found no significant differences between methods for curd fines, curd firmness and dressing retained (Tables 3 and 4b). Only curd made by the direct-set method from 3.1% protein-skim milk retained more dressing ($p < .05$) when 1.25 times the

TABLE 3. *Analysis of variance comparing recovery of protein and properties of curd by the two methods and at the three protein concentrations.*

Source of variation	d.f.	Mean squares						
		Protein (%) recovered in				Curd properties		
		Whey	1st wash	2nd wash	Curd	Size[1] (%)	Fines[2] (%)	Firmness[3]
Protein	2	26.7**	.067	.859	15.950	12.72	.197	1.9×10^4**
Rep/prot.	24	2.7	1.422	.333	6.601	7.160	.111	1.7×10^3
Method	1	10.1*	2.003	.254	3.894	68.07**	.002	2.8×10^2
Methods × Protein	2	5.6	.987	.189	2.321	5.698	.130	1.2×10^3*
Residual	24	1.8	.635	.281	3.943	8.532	.057	2.9×10^2
		Means[4]						
Methods								
Culture		15.90	3.30[a]	1.92[a]	77.99[a]	8.09	.62[a]	94.4[a]
Direct-set		15.04	3.68[a]	1.78[a]	78.53[a]	5.85	.63[a]	99.7[a]
		(.832)[5]	(.447)	(.298)	(1.115)	(1.64)	(.134)	(9.57)
Protein (%)								
3.1		16.85	3.55[a]	2.02[a]	77.52[a]	6.70[a]	.51[a]	135.2
3.5		15.01[a]	3.43[a]	1.60[a]	77.94[a]	6.30[a]	.70[a]	76.6[a]
3.9		14.55[a]	3.49[a]	1.92[a]	79.32[a]	7.92[a]	.67[a]	79.3[a]
		(1.129)	(.820)	(.397)	(1.769)	(1.843)	(.228)	(89.6)

*Significant at 5%

**Significant at 1%.

[1]Curd retained by 12.7 mm + 1.4 mm sieves.

[2]Whey and 1st + 2nd wash fines. Percent yield lost as curd fines.

[3]Curd firmness = shear value, kg per 100 g curd.

[4]Means not significantly different at the 1% level are joined by a common letter.

[5]$LSD_{.05}$ are bracketed.

TABLE 4a. *Comparison of means of nine replications for the following combinations of treatments.*

	3.1% Protein		3.5% Protein		3.9% Protein	
	Culture	Direct-set	Culture	Direct-set	Culture	Direct-set
YIELD						
1. Kg curd/100kg skim milk	14.04	14.68	16.48	17.10	18.71	18.76
2. Kg curd/kg protein	4.52	4.69	4.72	4.90	4.81	4.83
3. Kg curd/kg casein	6.14	6.45	6.24	6.47	6.31	6.32
4. Kg curd/kg	1.60	1.67	1.78	1.84	1.92	1.92
TOTAL SOLIDS DISTRIBUTION	(% Recovery)					
Less – GDL_1		53.43		47.37		49.13
1. Whey	49.92		44.65		40.93	
+ GDL_2		49.50		44.57		44.78
Less – GDL_1		13.26		13.52		11.58
2. 1st wash	8.80		9.05		10.20	
+ GDL_2		12.27		12.03		10.64
Less – GDL_1		6.40		5.56		5.76
3. 2nd wash	4.84		4.42		4.94	
+ GDL_2		5.93		5.15		5.26
Less – GDL_1		33.45		36.85		38.50
4. Curd	31.94		35.69		38.39	
+ GDL_2		30.98		34.22		34.44

	$^{a}LSD_{.05}$	$^{b}LSD_{.05}$
Kg curd/100g skim milk	.389	.631
Kg curd/Kg protein	.119	.169
Kg curd/Kg casein	.141	.208
Kg curd/Kg solids	.036	.060

[a] LSD's for comparing methods (culture vs direct-set) *within* protein %.
[b] LSD's for comparing protein % *within* each method.

TABLE 4b. *Comparison of means of nine replications for the following combinations of treatments.*

	3.1% Protein		3.5% Protein		3.9% Protein	
	Culture	Direct-set	Culture	Direct-set	Culture	Direct-set
PROTEIN DISTRIBUTION	(% Recovery)					
1. Whey	17.84	15.86	15.43	14.59	14.43	14.67
2. 1st wash	3.22	3.88	3.10	3.76	3.57	3.41
3. 2nd wash	2.11	1.92	1.56	1.64	2.08	1.77
4. Curd	77.11	77.92	77.40	78.48	79.46	79.18
CURD SIZE DISTRIBUTION	(%)					
1. 1.4 mm (1/18")	5.26	4.03	3.13	2.62	3.72	3.12
2. 2.83 mm (1/9")	67.50	67.91	55.23	56.90	52.21	60.58
3. 6.35 mm (1/4")	25.04	26.14	37.61	37.65	37.44	33.50
4. 12.7 mm (1/2")	2.18	1.91	4.02	2.83	5.96	3.03
1&4. 12.7 mm (1/4")	7.44	5.95	7.16	5.45	9.69	6.15
LOSSESS AS CURD FINES (%)	.529	.485	.602	.807	.732	.605
CURD FIRMNESS[1]	140.4	130.0	74.2	78.9	68.5	90.1
DRESSING RETENTION	(%)					
Normal[2]	84.4	92.0	—	—	—	—
1.25 × Normal	73.2	90.5	—	—	—	—
1.5 × Normal	—	—	86.7	89.2	81.8	75.8
2 × Normal	—	—	68.8	65.6	64.7	61.1

[1] Curd Firmness = shear value, kg/100g curd.
[2] Normal = 44 grams dressing/100 grams curd.

	$^{a}LSD_{.05}$	$^{b}LSD_{.05}$
Whey	1.305	1.459
1st wash	.775	.987
2nd wash	.516	.539
Curd	1.932	2.234
Fines	.232	.282
Firmness	16.6	30.7

[a] LSD's for comparing methods culture vs Direct-set *within* Protein %.
[b] LSD's for comparing protein % *within* method.

normal amount (44 g of 14% fat dressing/100 g of curd) was used.

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Rapid Method for Detection and Quantitation of Lipid Soils on Food Contact Surfaces: Evaluation of a Model System

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ABSTRACT

A rapid method for detection and quantitation of lipid-containing food soils on food-contact surfaces has been developed to ascertain whether these surfaces have been properly cleaned. The method is based on transfer of lipid-based soils from a food-contact surface to a polyethylene film and subsequent quantitation of the lipid, at 1750 cm^{-1}, by infrared spectrophotometry. Peak height at 1750 cm^{-1} is linearly related to the quantity of lipid on the polyethylene surface. Standard curves for peak-height against lipid distribution on the polyethylene film were constructed for stainless steel, glass and three types of plastic cutting board material (high density polyethylene, smooth nylotrol and rough nylotrol).

In the food industry, processing equipment and other food contact surfaces must be cleaned and sanitized after use. Proper cleaning must bring about complete removal of all food soils and residues from the surface and sanitation must reduce the bacterial numbers to acceptable levels. For proper sanitation to occur, close contact must exist between the food contact surface and sanitizer (*2*). Food-based soil residues on a surface are considered undesirable because the sanitizers will react with the food-soil residue and be less effective in reducing bacterial numbers. Further, the soil residues, if heavy enough, will shield bacteria from the effects of sanitizers so that bacterial numbers are not reduced. Since lipid residues are one of the most difficult soils to remove from surfaces, a method to ascertain whether these residues are present on a surface would be desirable for determining how well that surface has been cleaned before sanitizing.

Several methods for quantitating the amount of soil on food-contact surfaces have been developed. The surface film displacement method (*5*) and extraction and analysis of cleaning solutions after use (*4*) have been developed by Maxcy and Arnold. ^{14}C-labelled lipids were used by Harris and Satanek (*1*) while ^{32}P-labelled milk proteins and bacteria were used by Peters and Calbert (*6*). Leenerts et al. (*3*) developed a method based on light transmittance through glass surfaces before and after washing. Although some of these methods have been used successfully to determine soil on surfaces, none of them may be used in food processing establishments due to the presence of either radioactive isotopes or toxic, flammable solvents. The object of this research was to develop a rapid, reliable method of quantitating lipid-based soils on food-contact surfaces for use in all segments of the food industry. The method described in this paper is based on transfer of lipid-based soils from the food contact surface onto polyethylene film and subsequent quantitation of the lipid by infrared spectrophotometry.

MATERIALS AND METHODS

The film used to pick up the lipid from the food contact surface was 2.75 mil clear polyethylene (Promotional Packaging Products, Mississauga, Ontario) cut into 5-cm × 8-cm strips.

To transfer the soil from the food contact surface to the polyethylene film, a hand operated roller, built at the University of British Columbia specifically for this project, was employed. The roller, constructed of stainless steel (8.5 cm long × 6.4 cm diameter), and the attached wooden handle weighed 2.49 kg. A 2-cm wide strip of masking tape was wound several times around the center circumference of the roller so that the entire weight of the roller was concentrated on this 2-cm wide area (Fig. 1). The design of the roller is such that the operator does not apply pressure to the roller but merely guides it back and forth across the polyethylene film a specific number of times. This ensures that the force applied to the polyethylene film is constant from sample to sample.

Food contact surfaces tested included stainless steel, glass (microscope slides, Fisher Scientific Ltd., Fairlawn, N.J.), nylotrol plastic cutting surface (Raimac Industries Ltd., Vancouver, B.C.) and high density polyethylene (HDPE) cutting surface (Cadillac Plastic, Vancouver, B.C.). The surface of nylotrol was dull and pebbly, whereas the surface of HDPE was smooth and shiny. A fifth food contact surface tested was nylotrol which had been hand-sanded to a smooth surface with 20 grit and wet-dry sandpaper to eliminate the effects of the irregular surface of nylotrol. The area of each piece of food contact surface, approximately 30 cm^2, was accurately determined with an electronic planimeter (Numonics Corp., North Wales, PA). Before use, the test surfaces were repeatedly scrubbed in hot water containing dishwashing detergent so that no residual fat remained on the surfaces. After washing, the pieces were thoroughly rinsed with water and then carefully dried with a paper towel.

The surfaces were artificially soiled, with commercial lard (Maple Leaf Tenderflake Lard, Canada Packers Ltd., Toronto, Ont.), using a cotton swab. The lipid was spread evenly over the surface of each sample and the amount of lipid applied was determined gravimetrically. The density of soiling (mg lipid/cm^2surface) was then calculated for each sample. Each lipid/surface combination was permitted to equilibrate at least 10 min at 20 C before analysis.

A piece of polyethylene film was placed on each sample of soiled surface. The roller was placed on the polyethylene and moved back and forth across the same area of the film 10 times, so that a 2-cm × 4-cm area of the polyethylene film - food contact surface interface was covered. The polyethylene film was removed from the food contact surface and mounted in a cardboard sample holder (5 cm × 8 cm) which had a 1-cm × 3-cm window cut in the center of it. This polyethylene film was aligned so that the 2-cm × 4-cm lipid containing area was centered in the window of the sample holder. The sample and

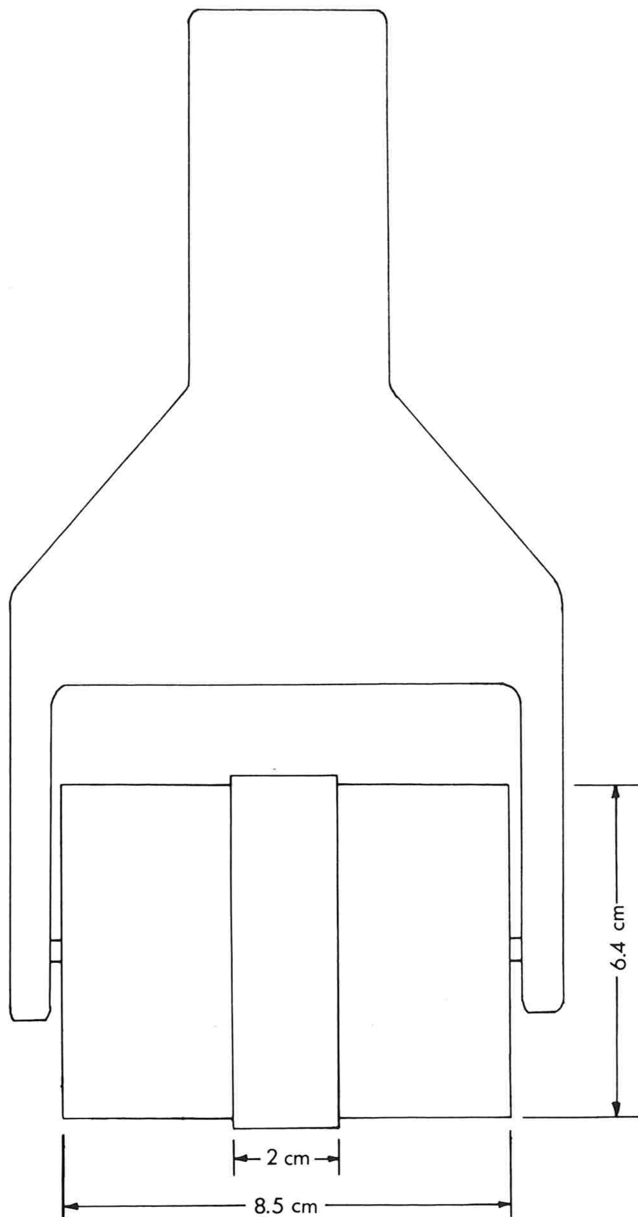

Figure 1. *Stainless steel hand roller used to transfer lipid from food contact surfaces to the polyethylene film.*

holder were placed in the sample beam of a Perkin-Elmer Model 457 Grating Infra Red Spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) and the spectrum was determined against air. A scan was started at 2000 cm^{-1} and terminated at 1600 cm^{-1}. The spectrophotometer was operated on fast scan so that exactly 64 sec elapsed between the time the sample was placed in the sample beam and the time the absorption band was at its maximum at 1750 cm^{-1}. This time interval is critical since at higher concentrations, the lipid tends to melt off the polyethylene film due to heat picked up from the infrared beam. As a consequence, peak-height would decrease with time.

A baseline was drawn across the bottom of the peak and the peak-height was measured. Each peak-height measurement was paired up with the soiling density (mg lipid/cm^2) for that surface. A simple linear regression analysis through the origin was performed for each food contact surface to develop the standard curves (*7*).

RESULTS AND DISCUSSION

After applying pressure to the polyethylene film on a lipid-soiled food contact surface, the lipid was transferred from the food contact surface to the polyethylene film (Fig. 2). Animal and plant lipids consist of mixtures of triglycerides which contain the C = 0 residue. This structure exhibits a strong absorption band around 1750 cm^{-1} of the infrared spectrum (Fig. 3a). Since polyethylene is free of C = 0 residues, an absorption band at 1750 cm^{-1} was not detected when polyethylene alone was examined (Fig. 3b). Thus the entire absorption band, or peak, at 1750 cm^{-1} is due to the lipid transferred from the food contact surface to the polyethylene.

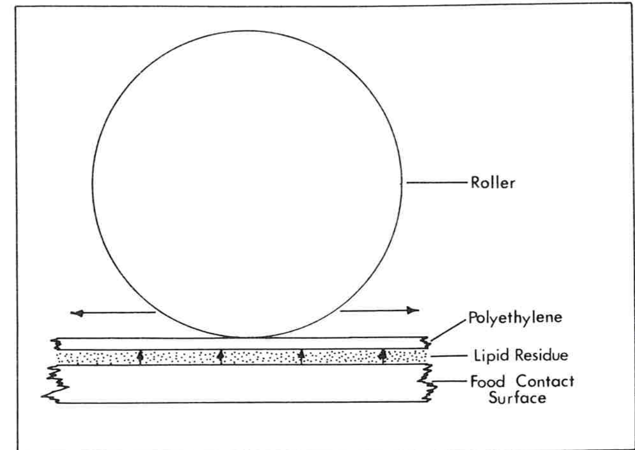

Figure 2. *Transfer of lipid from a food contact surface to polyethylene film with the aid of the stainless steel roller.*

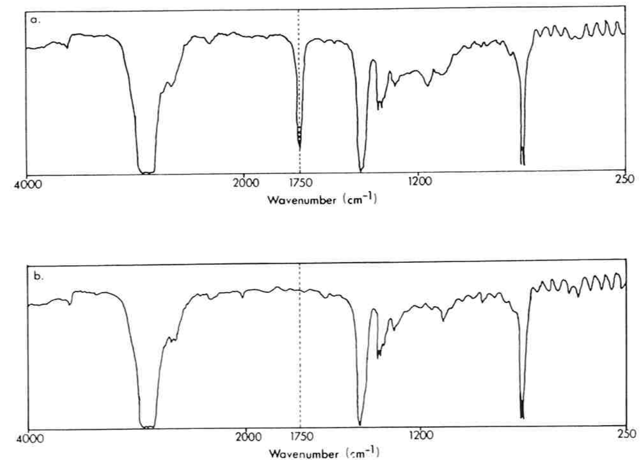

Figure 3. *a. Infrared spectrum of polyethylene film and lipid (lard). b. Infrared spectrum of lipid-free polyethylene film.*

Of the numerous plastic films tested, polyethylene was found to be the best film to use for this purpose as it had a window in the region of 1600 to 2000 cm^{-1}. Other films had interfering absorption bands in this region which would not have permitted accurate measurement of the C = 0 absorption band.

Peak-height at 1750 cm^{-1} is linearly related to the quantity of lipid present on the food contact surface. This permitted development of standard curves for each of the food contact surfaces tested (Fig. 4). The measurement of peak-height was equally reliable as peak area measurements for quantitation of lipid on food contact surfaces. Each standard curve was linear only up

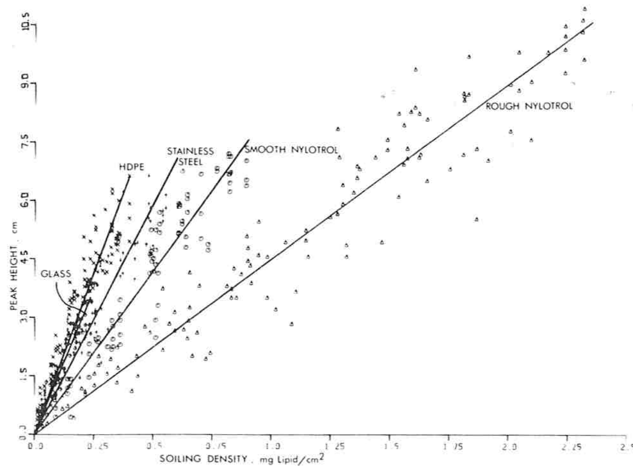

Figure 4. *Standard curves for five food contact surfaces: HDPE (n = 111; r^2 = 0.90); glass (n = 47; r^2 = 0.90); stainless steel (n = 94, r^2 = 0.89); smooth nylotrol (n = 78, r^2 = 0.90); rough nylotrol (n = 118, r^2 = 0.92).*

to a limiting concentration of lipid, beyond which the increase in peak-height was minimal with increasing soiling density.

The various parameters characterizing the linear portion of the standard curves for the various surfaces tested are summarized in Table 1. The r^2 values were all fairly good with the best correlation occurring with rough nylotrol and the poorest with stainless steel.

The parameters obtained from developing the standard curves provided additional information on certain characteristics of the food contact surfaces examined. For the samples with smooth surfaces (stainless steel, glass, HDPE, smooth nylotrol), the coefficient or slope of the standard curve gives an indication of the affinity of that surface for lipid. With a soiling density of 0.2 mg of lipid/cm², a peak height of 3.3 cm was produced from the HDPE surface, whereas a peak height of only 1.6 cm was produced from the smooth nylotrol. This means that more lipid was transferred to the polyethyene film from the HDPE surface than from the smooth nylotrol surface under identical conditions. Thus the smooth nylotrol surface had a greater affinity for lipid than the HDPE surface. It appears then, that the smaller the slope of the line, the greater is the affinity for lipid by that surface.

For the rough nylotrol sample, physical entrapment of the lipid by the irregular features of this surface will take place. This is illustrated by examining the peak-heights produced by the 0.2-mg of lipid/cm² soiling density on both smooth and rough nylotrol surfaces. A peak-height of 1.6 cm was produced for smooth nylotrol, while a peak height of only 0.9 cm was produced with rough nylotrol, indicating that more lipid was being transferred to the polyethylene film from the smooth nylotrol than from the rough nylotrol under identical conditions. Thus physical entrapment of the lipid will occur on an irregular surface.

Knowing the affinity of a certain surface for lipid and whether it entraps soil in surface irregularities will enable

TABLE 1. *Parameters of standard curves for five food contact surfaces.*

Surface	Coefficient[1]	r^2	Limit of linearity (mg lipid/cm²)	Peak height (cm) from 0.2 mg lipid/cm²
Nylotrol				
Rough	4.5	0.92	2.35	0.9
Smooth	8.0	0.90	0.90	1.6
Stainless				
Steel	12.0	0.89	0.60	2.4
Glass	15.0	0.90	0.24	3.0
HDPE	16.5	0.90	0.40	3.3

[1]Equation of Line, y = ax, a = coefficient, y = peak height (cm), x = soiling density (mg lipid/cm²).

a food company to choose the most practical food contact surface for its needs. Thus if a specific process contaminates a surface with lipid-based soils, a food contact surface with a low affinity for lipid should be chosen for easy clean-up. It is also generally desirable to choose a food contact surface that is smooth rather than one with surface irregularities since it is difficult to remove soils entrapped in these irregularities.

The method developed in this paper may also be useful for other purposes. The ease of cleaning of various food contact surfaces may be determined by using this method to quantitate the amount of lipid-based soil remaining after a given cleaning procedure. This method may also be used to test the efficiency of specific detergents or detergent-sanitizers by determining lipid residues on a surface after different cleaning procedures. Also, the degree of soiling from a certain process may be examined with this method.

It should be noted that all work was done at 20 C so that the standard curves developed are only for surfaces that have a temperature of 20 C. New standard curves would have to be developed for surfaces having different temperatures since the properties of lipid change with temperature. Also, the method described here would be suitable for use only on horizontal, flat surfaces. By incorporation of a suitable tensiometer in the handle, however, verticle and even sloping surfaces could also be sampled by keeping the force of the roller, perpendicular to the surface being sampled, at a constant value.

The effects of other food constituents such as water, protein, carbohydrates and minerals on this method have not been evaluated since a model system, where pure lard was used to soil the food contact surfaces, was employed in this study. Work is currently in progress to evaluate the effects of other food constituents on the reliability and sensitivity of this method of determining lipid residues on food contact surfaces.

Two other methods of transferring the lipid from the food contact surface to the polyethylene film were evaluated. The first method involved dropping a 1-kg weight from a height of 10.5 cm onto the polyethylene film which had previously been placed on the soiled cutting board. There was a transfer of lipid to the polyethylene; however, this method was abandoned because the force used was such that it could have

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Inhibition of Botulinum Toxin Formation in Bacon by Acid Development

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ABSTRACT

Lactobacillus plantarum, as a producer of lactic acid, and sucrose as a fermentable carbohydrate were evaluated for use in lowering the amount of or eliminating sodium nitrite in bacon. This work was limited to effect on antibotulinal properties. Organoleptic effects were not considered. Slices of bacon were inoculated with spores of *Clostridium botulinum* types A and B with or without simultaneous inoculation with a culture of *L. plantarum*, vacuum-packaged and incubated at 27 C. Samples were taken after various periods of incubation and assayed for botulinal toxin. We found that (*a*) sodium nitrite alone, at 120 ppm, did not give bacon extended protection against development of botulinum toxin if a fermentable carbon source (sucrose in these instances) was not present; (*b*) without added lactic acid bacteria, the effectiveness of 120 ppm of sodium nitrite plus sugar was variable and depended upon growth of naturally contaminating bacteria and (*c*) lactic acid bacteria with an adequate amount of sucrose gave good protection against development of botulinal toxin. Upon temperature abuse, acid was produced and growth of *C. botulinum* was inhibited. Because the protective properties against development of botulinal toxin in the sugar-lactic acid bacteria system were not dependent on the presence of nitrite, nitrite can be lowered to the level necessary to make organoleptically acceptable products without sacrificing safety, thus less nitrosamine formation may be achieved.

It is well known that sodium nitrite (nitrite) adds antibotulinal protection to cured meat (*4,9,12,14*). It has also been shown that nitrosamines (nitrosopyrrolidine and dimethyl nitrosamine) can be formed when bacon is cooked in certain ways (*10,15,17*) through reactions of nitrite with secondary amines. Reducing the nitrite content of bacon will minimize nitrosamine formation (*17*), but will increase the likelihood of botulinal toxin formation if bacon is subjected to temperature abuse.

Botulinal toxin formation is influenced by pH, water activity, salt, nitrite, inoculum level and temperature (*3,4,5,8,14,16*). In many types of foods, including bacon, these factors cannot be manipulated much without affecting safety and organoleptic quality. To reduce nitrite without sacrificing safety, therefore, development of a new system has become necessary.

A series of inoculated pack experiments at the Food Research Institute led to two basic observations: (*a*) toxicity development in inoculated bacon was erratic and (*b*) non-toxic samples had pH levels of 5.0 or less. We speculated that the naturally contaminating bacteria in bacon grew upon incubation and produced acid from sugar, thus lowering the pH and preventing the growth of *Clostridium botulinum*. The effect of lactic acid bacteria and sugar for preventing botulinal toxin formation has been reported for fermented sausage (*6*). Because of the above observations it was felt that botulinal toxin production could be consistently inhibited if a controlled level of lactic acid bacteria was added instead of depending on natural flora. It was anticipated that only slight acid formation would take place unless the product was subjected to temperature abuse. Experiments were initiated to explore this possibility.

We learned early in the work that the American Bacteriological and Chemical Research Corporation (ABC Research Corp., Gainesville, FL) had developed a procedure to lower residual nitrite in bacon by controlling pH with lactic acid bacteria. The effect of this procedure on antibotulinal properties was at that time unknown.

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MATERIALS AND METHODS

Bacon

Bacon was produced in the Meat Science Laboratory of the University of Wisconsin using bellies from locally slaughtered animals. Bacon was also obtained from four commercial plants.

Pork bellies treated in the Meat Science Laboratory were pumped to the following composition: 550 ppm (mg/kg) of sodium ascorbate, 1.55% of sodium chloride and 0.31% of sodium tripolyphosphate (STPP), unless otherwise indicated. Sucrose and nitrite were varied as experimental conditions specified. One experimental series had 0.26% of potassium sorbate (sorbate) added.

The bellies were pumped with pickle solution to a 10% weight gain, giving the above composition. Extent of variation in pumping is shown by sodium chloride values (cf. Table 2). They were then held in a plastic bag at 2-4 C for 22 h to obtain best distribution of the pumped pickle, drained by hanging for 2 h, smoked at 38 C for 1.5 h, then held at 58 C with 40% relative humidity until the internal temperature reached 55 C. The bellies were then chilled by hanging overnight at 2-4 C, sliced into 20-25-g slices and stored at 4 C in plastic bags. Residual nitrite values obtained by this procedure fall within normal commercial practice limits. The sliced bacon was inoculated with *C. botulinum* spores, and in some instances with *Lactobacillus plantarum* cells, and vacuum-packaged within 28 h after slicing. In samples from one commercial operation, the lactobacilli were added at the pumping stage and went through the subsequent smoking and heat treatment comparable to that used at our Meat Science Laboratory.

Bacterial cultures

Five strains each of *C. botulinum* types A (56 A, 62 A, 69 A, 77 A, 90 A) and B (53 B, 113 B, 213 B, 13983 B and Lamanna-okra B) were used. Each strain was grown in a manner conducive to sporulation (*7*). Spores were harvested by centrifugation and washed with sterile water. The washed spores were sonicated briefly to destroy vegetative cells and sporangia (30 sec at a time, a maximum total sonication time of 5 min). During the sonication process, spores were kept in a sodium chloride-ice bath. The sonicated spores were thoroughly washed with sterile water using centrifugation, then kept frozen in small portions until used. Each frozen spore suspension was thawed and enumerated. Suitable dilutions with sterile water were combined to produce a mixed inoculum consisting of approximately equal numbers of each strain.

A frozen culture of *L. plantarum* was obtained from ABC Research Corp. The frozen culture was thawed in a water-bath at room temperature and used immediately upon suitable dilution in a 4% sodium chloride solution with *C. botulinum* spores.

Antitoxin

C. botulinum anti A/B toxin was obtained from Connaught Laboratories, Toronto, Canada.

Enumeration of C. botulinum

C. botulinum was enumerated by the five-tube Most Probable Number (MPN) method using trypticase-peptone-glucose-yeast extract medium (*11*). Tubes showing bacterial growth were tested for the presence of botulinal toxin by the mouse test (*11*). Only toxin-positive tubes were counted as growth-positive in determining the MPN of *C. botulinum.*

Inoculation

All samples were inoculated with *C. botulinum* at 1×10^3 spores per gram, and *L. plantarum* at approximately 4×10^6 cells per gram. Four slices of bacon, selected randomly from different bellies or different parts of a belly, comprised one sample. For inoculation, the slices were spread flat on paper and each slice received one drop of a 4% sodium chloride solution containing the 10-strain mixture of *C. botulinum* spores, and the lactic acid bacteria.

The inoculum was spread by hand with a plastic glove, then the slices were rolled together and vacuum-packaged (Vacuum Sealer, Packaging Aids Corporation, San Francisco, CA) in an oxygen-impermeable film (Saran coated Mylar, Curwood, Inc., New London, WI).

Incubation

The inoculated and vacuum-packaged samples were incubated up to 8 weeks at 27 C. In one experiment, they were stored at 4 C for 3 weeks and then incubated at 27 C.

Extraction and assay of botulinal toxin

The contents of a vacuum package were transferred into a Stomacher bag, weighed and an equal volume of gel-phosphate buffer, pH 6.2 (*11*), was added (or double distilled water for pH determination). The sample was then blended using a Stomacher (Model 400, Cooke Laboratory Products, Alexandria, VA) for 2 min. After blending, the contents of the bag were filtered through cheese cloth into a beaker. A portion of the filtrate was centrifuged at $5{,}000 \times g$ for 5 min, and the supernatant fraction was tested for botulinal toxin.

For detection of toxin, each of two mice was injected intraperitoneally with 0.5 ml of the extract from the test sample. The mice were held up to 4 days and examined for symptoms and death characteristic of *C. botulinum* poisoning. When death occurred, two additional mice were challenged with a sample-antitoxin mixture which was preincubated at 37 C for 30 min (*11*). Unneutralized extract was again injected to two more mice as a control.

Other analyses

The pH was determined on 1:1 (w/v) double distilled water extracts, using a Beckman pH meter (Model 3500) with a Futura combination electrode. Sodium chloride and moisture were determined by AOAC methodology (100-102 C, 16-18 h) (*1*). Nitrite was determined by the revised AOAC method (*2*). All determinations were at least in duplicate.

Aerobic plate count was done using APT-agar plates (*13*) incubated at 30 C for approximately 20 h.

RESULTS

The results of toxin assay in our first experiment are shown in Fig. 1. The bacon used in this experiment was made at a commercial plant with the same target composition as described in Materials and Methods. All the products contained 0.9% sucrose.

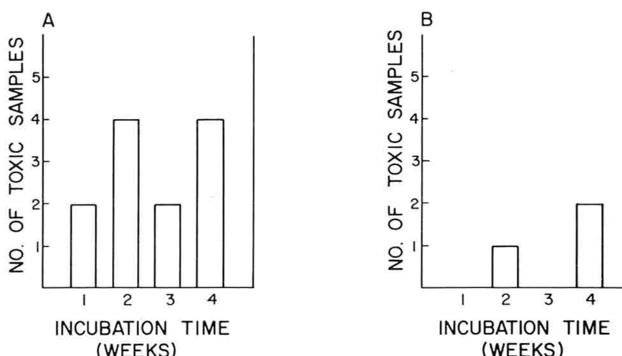

Figure 1. *Effect of sodium nitrite on toxin production in bacon by* Clostridium botulinum. *Five samples were tested for toxicity at each sampling period. Open columns indicate number of toxic samples found at each sampling period. Panel A shows the results with 80 ppm of nitrite and Panel B with 120 ppm nitrite. Both products contained 0.9% of sucrose, but no added lactic culture.*

Panel A shows the results obtained using bacon with 80 ppm of nitrite. Toxic samples appeared as early as 1 week after the start of the incubation although not all the samples became toxic upon further incubation.

Panel B shows the results with 120 ppm of nitrite. A total of three toxic samples appeared out of 20 tested in 4 weeks of incubation. With no nitrite (data not shown), all the samples tested were found toxic after 1 week.

Although the inhibitory effect of nitrite on botulinal toxin formation was apparent, patterns of toxin formation with 80 ppm of nitrite were irregular, i.e., 2 out of 5 in 1 week, 4 of 5 in the second week, 2 of 5 in the third week and 4 of 5 in the fourth week. Irregular development of toxicity was also seen with 120 ppm nitrite; the most striking observation being that 120 ppm of nitrite did not give reliable prevention of toxicity development, with only the natural inoculum present.

Another experiment (data not shown) with 60 ppm of nitrite and 0.9% of sucrose produced only one toxic sample out of a total of 100 tested through 6 weeks of incubation. The pH values ranged from 4.4 to 5.0 in the non-toxic samples after 6 weeks of incubation.

The results of these experiments suggested that something other than nitrite was influencing botulinal toxin development, and this other factor could be a rapid drop in pH. It seemed likely that pH was dependent on the extent of natural inoculation with lactic organisms plus the availability of fermentable carbohydrate. If a product was relatively lightly inoculated, pH would decrease slowly and toxin would develop. If a heavily

inoculated product did not have fermentable carbohydrate, this also would limit acid production and pH drop. Finally, if a heavily inoculated product had fermentable carbohydrate available, a rapid and deep drop in pH could be expected.

These observations led us to the consideration of lactic acid bacteria as an "artificial contaminant" which should reduce the pH of bacon rapidly and hence inhibit botulinal toxin production upon temperature abuse.

Figure 2 shows effect of variation in nitrite (0 and 120 ppm), sugar (0 and 0.9%) and lactobacilli [added (+) and not added (−)].

Panels A and B show that lactobacilli or sucrose alone, with no nitrite, did not inhibit toxin formation. While a substantial drop in pH occurred when sucrose was used alone (Panel B), the rate of the pH decrease was not as rapid as in samples where lactobacilli and sucrose were used together. With sucrose alone, the pH reached 5.9 in 2 days, 5.5 in 4 days and 4.9 in 1 week and all samples were toxic in 1 week.

Panel C shows the results obtained when sucrose and lactobacilli were used together without nitrite. The pH reached 5.4 in 2 days and 5.0 in 4 days, and no toxic samples were found.

Panels D and E show the results obtained with 120 ppm of nitrite without added sucrose, with and

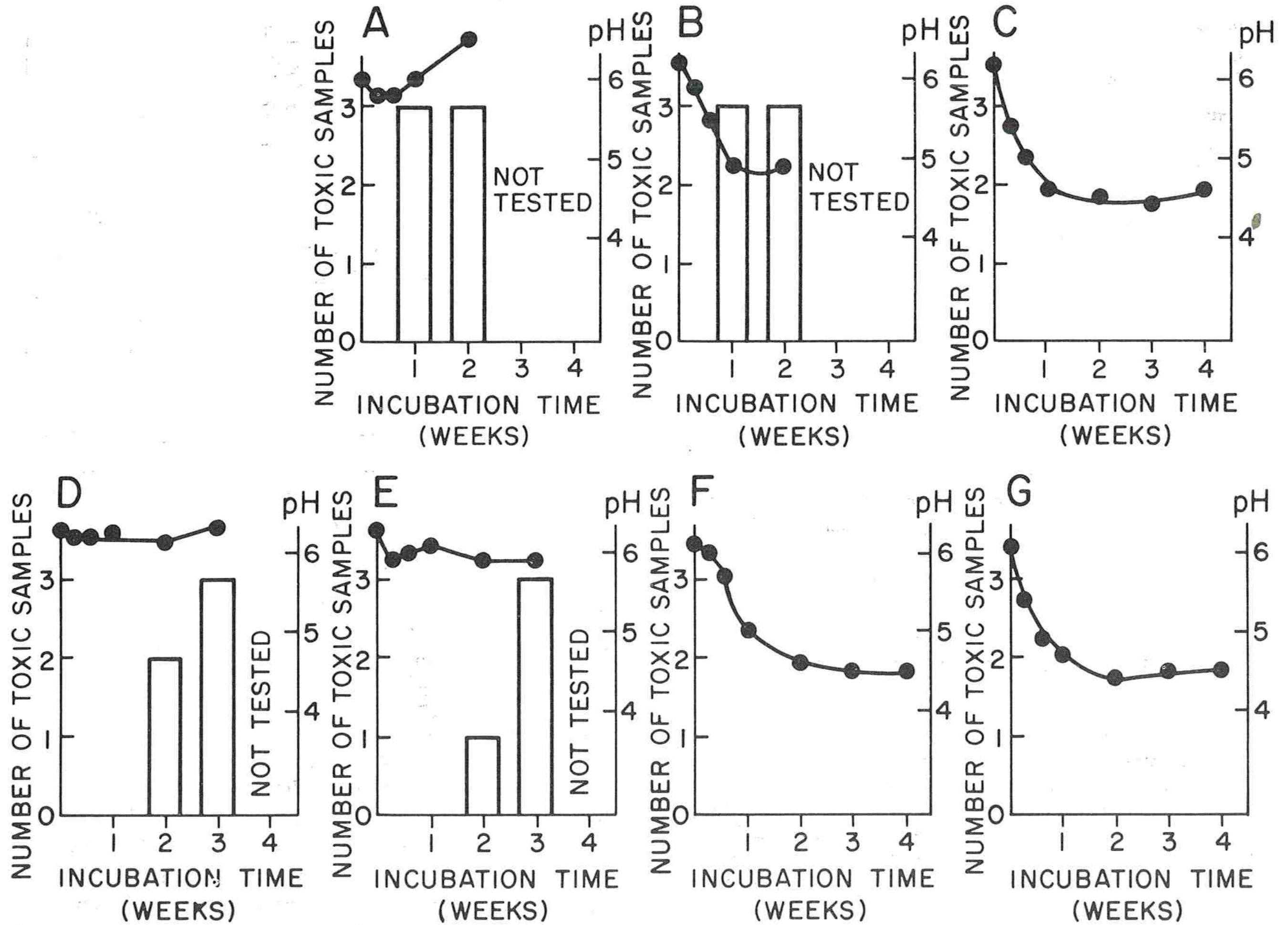

Figure 2. *Effect of sodium nitrite, sucrose and* L. plantarum *on toxin formation by* C. botulinum *and pH in bacon. Three samples were tested for toxicity at each sampling period. Open columns indicate number of toxic samples found at each sampling period. Closed circles indicate pH values determined. The pH values are listed below.*

Panel	Variables: $NaNO_2$ (ppm)	Sucrose (%)	*L. plantarum* inoculated
A	0	0	+[a]
B	0	0.9	−[b]
C	0	0.9	+
D	120	0	−
E	120	0	+
F	120	0.9	−
G	120	0.9	+

[a]+, inoculated; [b]−, uninoculated.

	pH at Incubation days or weeks (at 27 C)						
	Days			Weeks			
Panel	0	2	4	1	2	3	4
A	6.0	5.8	5.8	6.0	6.5	nt[c]	nt
B	6.2	5.9	5.5	4.9	4.9	nt	nt
C	6.2	5.4	5.0	4.6	4.5	4.4	4.6
D	6.3	6.2	6.2	6.3	6.1	6.3	nt
E	6.3	5.9	6.0	6.1	5.9	5.9	nt
F	6.1	6.0	5.7	5.0	4.6	4.5	4.5
G	6.1	5.4	4.9	4.7	4.4	4.5	4.5

[c]Not tested.

without added lactobacill. Toxin formation was delayed but not eliminated. Toxic samples appeared in 2 weeks. These samples showed little pH decrease.

Panel F shows that 120 ppm of nitrite with 0.9% of sucrose without added lactobacilli prevented toxin formation with a substantial drop in pH. It is likely in this group that the natural contamination of the bellies provided enough lactic acid organisms to bring about lowering of the pH.

Panel G shows the effect of 120 ppm nitrite, 0.9% sucrose and added lactobacilli. The pH drop was more rapid than in Panel F, and no toxic samples developed.

Figure 3 shows effect of 21 days of refrigerated storage before incubation. In this series, the same group of products was used as in Fig. 2. This group of samples was held in refrigerated storage (4 C) for 21 days before incubation. There is a similar pattern of inhibition of toxin formation in the two groups.

Panel G, covering a group made with 120 ppm of nitrite, 0.9% sucrose and added lactobacilli, shows one toxic sample during the first week, and none thereafter. The pH values after refrigerated storage were generally about 0.1 to 0.3 unit lower than at the time of inoculation, though one sample (C) dropped 0.6 unit. During the 3-week refrigeration period residual nitrite values dropped almost to background levels in the

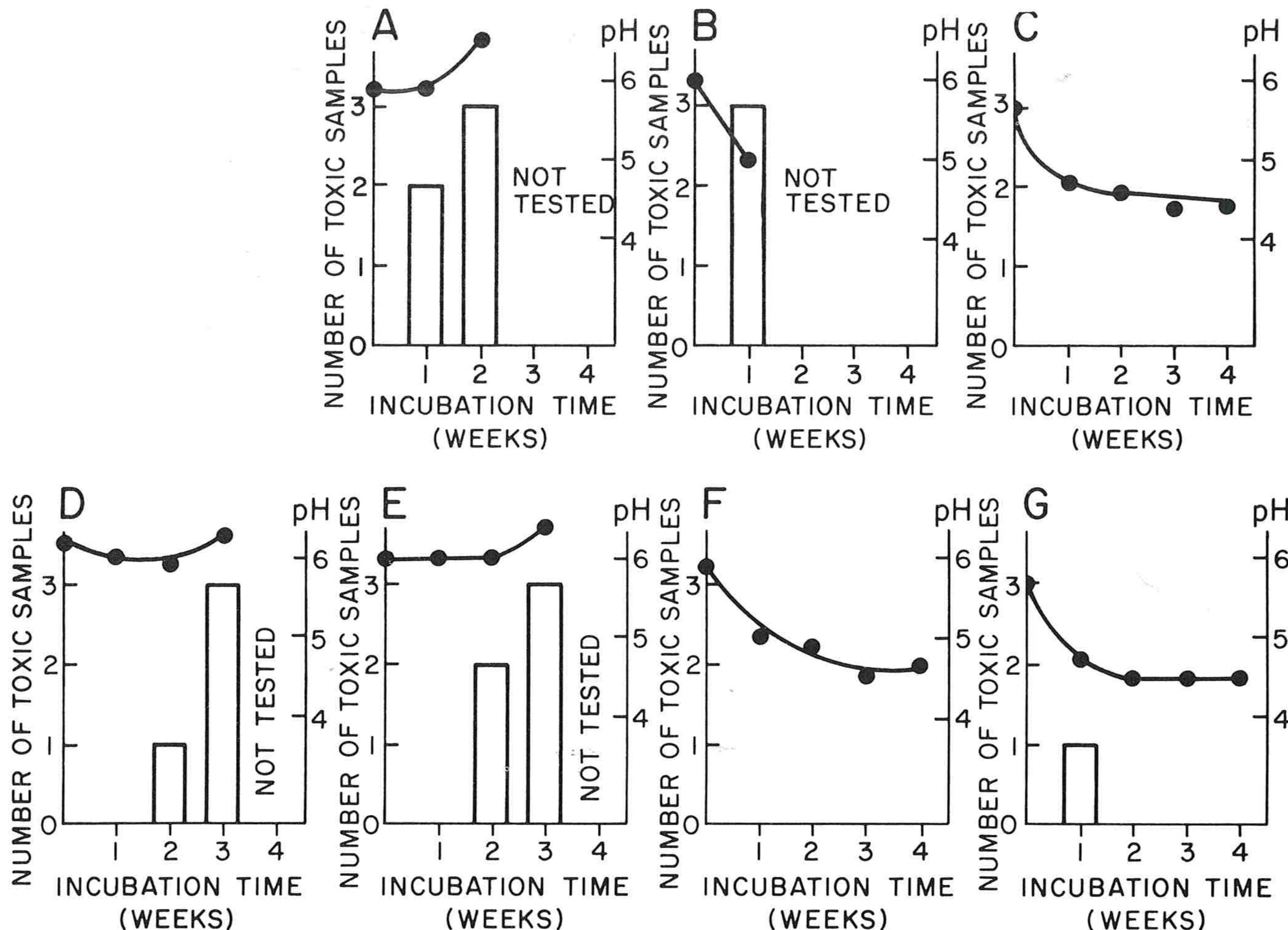

Figure 3. *Effect of sodium nitrite, sucrose and the inoculum of* L. plantarum *on toxin formation by* C. botulinum *and pH in bacon, and effect of 3 weeks of refrigerated storage. The inoculated and vacuum packaged bacon was stored at 4 C for 3 weeks then incubated at 27 C. The incubation time in the figure is the time after the 27 C incubation started. Three samples were tested for toxicity at each sampling period. Open columns indicate number of toxic samples found at each sampling period. Closed circles indicate pH values determined.*

The pH values are listed below.

Panel	Variables		
	$NaNO_2$ (ppm)	Sucrose (%)	*L. plantarum* inoculated
A	0	0	+[a]
B	0	0.9	–[b]
C	0	0.9	+
D	120	0	–
E	120	0	+
F	120	0.9	–
G	120	0.9	+

[a]+, inoculated; [b]–, uninoculated.

Panel	pH at incubation weeks (at 27 C)				
	0	1	2	3	4
A	5.9	5.9	6.5	nt[c]	nt
B	6.0	5.0	nt	nt	nt
C	5.6	4.7	4.6	4.4	4.5
D	6.2	6.0	5.9	6.3	nt
E	6.0	6.0	6.0	6.4	nt
F	5.9	5.0	4.9	4.5	4.6
G	5.7	4.7	4.5	4.5	4.5

[c]Not tested.

products made with added lactobacilli.

Figure 4 shows the effect of variation in sucrose level, and STPP. The amounts of sucrose tested were 0.1, 0.5 and 0.9%. To learn if the buffering effect of STPP would interfere with a decrease in pH, the 0.5%-sucrose batches were made with and without 0.31% of STPP. The 0.9%-sucrose batch was made with 0.31% of STPP. The 0.1%-sucrose batch did not contain STPP.

Panel A shows there was no toxicity in the bacon with 0.9% of sucrose and added lactobacilli. Panels B and C show no toxicity occurred in bacon made with 0.5% of sucrose and added lactobacilli, with and without 0.31% of STPP. There was no significant variation in pH between the two lots. Panel D shows toxicity developed by the third week in bacon made with 0.1% sucrose and added lactobacilli. The pH did not drop below 5.4.

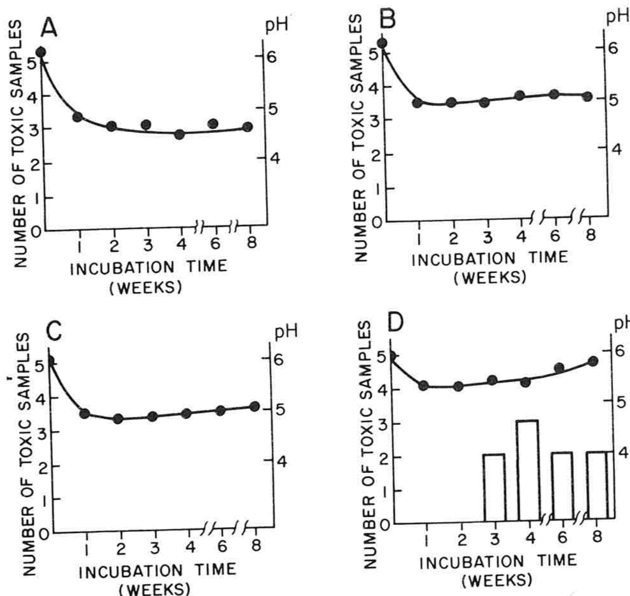

Figure 4. *Effect of variation in sucrose concentration and addition of STPP on toxin formation and pH in bacon inoculated with* L. plantarum *and* C. botulinum. *All the bacon was made with 120 ppm of sodium nitrite. Five samples were tested for toxicity for each lot at each sampling period. Open columns indicate number of toxic samples at each sampling period. Closed circles indicate pH values.*

Panel	Variables	
	Sucrose (%)	STPP (%)
A	0.9	0.31
B	0.5	0.31
C	0.5	0
D	0.1	0

The pH values are listed below.

Panel	pH at incubation weeks (at 27 C)						
	0	1	2	3	4	6	8
A	6.2	4.9	4.7	4.7	4.5	4.7	4.6
B	6.2	5.0	5.0	5.0	5.1	5.1	5.0
C	6.1	5.0	4.9	4.9	5.0	5.0	5.1
D	6.0	5.4	5.4	5.5	5.4	5.7	5.8

Figure 5 presents the effect of sorbate as well as sucrose and lactobacilli. This series was done to test the antibotulinal effectiveness of a combination of nitrite (40 ppm and 120 ppm) and sorbate (0.26%). Panel A, with 40 ppm of nitrite and no other additives, shows all samples toxic in 1 week. Little pH drop was observed.

Panel B, with 40 ppm of nitrite and 0.26% of sorbate, shows some inhibitory effect, though at the 6-week inspection, 3 of 5 samples were toxic.

Panel C, with 40 ppm of nitrite, 0.9% of sucrose and added lactobacilli, showed no toxicity through 6 weeks. A decrease of pH to 5.1 within 1 week was observed.

Panel D, with 120 ppm of nitrite and no other additives shows that 4 of 5 samples were toxic by 1 week. The pH did not drop below 6.0.

Panel E, with 120 ppm of nitrite and 0.26% of sorbate, shows there was an onset of toxicity by the third week. The pH did not drop below 6.0.

Panel F, with 120 ppm of nitrite, 0.9% of sucrose and added lactobacilli shows there was no toxicity. A decrease of pH to 5.1 was achieved in 1 week.

Table 1 summarizes the effect of variables on residual nitrite. Residual nitrite dropped very rapidly on incubation in all samples that became acidic, and by the fifth day were at 5 ppm or lower, whether they had started at 120 ppm or 40 ppm. Refrigerated samples showed a more gradual reduction.

Table 2 summarizes results obtained for initial *C. botulinum* count, salt, moisture and aerobic plate count (reflecting lactic acid bacteria). Generally, the *C. botulinum* count was higher than expected. This might involve an artifact resulting from the dilution factor used, i.e., the sample was diluted with an equal volume of gel-phosphate buffer, blended, filtered through cheese cloth and a 1-ml portion was taken for the successive 10-fold dilutions. The MPN obtained was then multiplied by two because of the first dilution with gel-phosphate buffer. If the distribution of the spores was even between the aqueous phase and solid phase, then the multiplication by 2 would be justified, but if spores were distributed more heavily in the aqueous phase, the factor 2 for the multiplication would make the number of spores obtained too large.

DISCUSSION

Table 3 summarizes the toxicity results obtained in the experiments in this series, plus data from previous relevant experiments not shown in the text. If one considers the data in terms of added nitrite, the following conclusions are evident.

No nitrite

When lactobacilli, but no sucrose, were added, toxin development was rapid, with 26 of 27 samples becoming toxic, almost all within 1 week. When 0.9% of sucrose was added, but no lactobacilli, practically all the samples became toxic in 1 week, and 50 of 52 samples were toxic by 2 weeks. When both 0.9% of sucrose and lactobacilli were added, only 1 sample of 49 became toxic. The toxic sample occurred during the fourth week of incubation, though the inspection at the fifth week showed no toxic samples.

40 ppm of nitrite

When no sucrose or lactobacilli were added, 47 samples of 50 became toxic. Addition of 0.9% of sucrose plus lactobacilli gave no toxic samples out of 30. It is evident that nitrite alone at 40 ppm was not effective.

120 ppm of nitrite

When neither sucrose or lactobacilli were added, there was some protective effect from nitrite alone, though 17 toxic samples occurred in 28 tested. Most of the toxic samples appeared by the second week of incubation, some as early as after 1 week. A similar trend was seen when lactobacilli, but no sugar, were added. Thirty four samples of 68 became toxic. When sucrose was added at a concentration between 0.5 and 0.9%, but no lactobacilli, substantial protection against toxin formation resulted, with 4 samples of 149 showing toxicity. This protection is apparently due to production of acid by naturally contaminating bacteria in the bacon. It is likely that 120 ppm of nitrite delayed growth of *C. botulinum*, thus giving the naturally contaminating bacteria time to grow and lower the pH. The reduction in

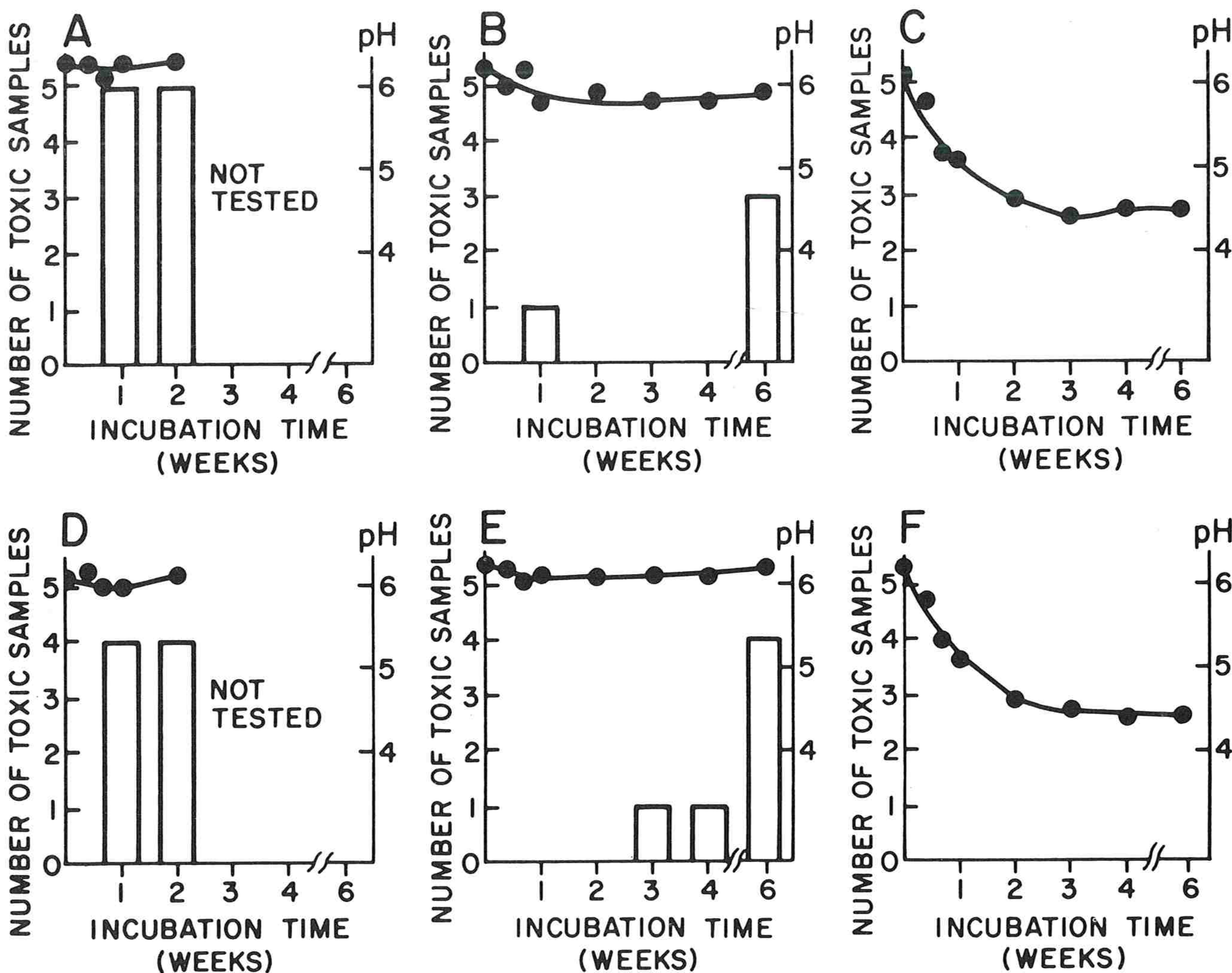

Figure 5. *Effect of sorbate and lactic acid bacteria-sucrose on toxin formation and pH in bacon inoculated with* C. botulinum. *Five samples were tested for toxicity for each lot at each sampling period. Open columns indicate number of toxic samples at each sampling period. Closed circles indicate pH values.*

Panel	Variables			
	$NaNO_2$ (ppm)	Potassium (%)	Sucrose (%)	*L. plantarum*
A	40	0	0	–[a]
B	40	0.26	0	–
C	40	0	0.9	+[b]
D	120	0	0	–
E	120	0.26	0	–
F	120	0	0.9	+

[a]–, not added; [b]+, added.

The pH values are listed below.

Panel	pH at Incubation days or weeks (at 27 C)							
	Days			Weeks				
	0	3	5	1	2	3	4	6
A	6.3	6.3	6.1	6.3	6.3	nt[c]	nt	nt
B	6.2	6.0	6.2	5.8	5.9	5.8	5.8	5.9
C	6.1	5.8	5.2	5.1	4.6	4.4	4.5	4.5
D	6.1	6.2	6.0	6.0	6.1	nt	nt	nt
E	6.3	6.2	6.1	6.1	6.1	6.1	6.1	6.1
F	6.2	5.8	5.3	5.1	4.6	4.5	4.4	4.4

[c]Not tested.

pH was not as rapid as in products inoculated with lactic acid organisms, but pH in most instances still ultimately dropped to well below 5.0. It appears there was competition between lactic organisms and *C. botulinum*, and if enough acid was produced early the product did not become toxic. When sucrose (0.5% or more) was added, with lactobacilli, there was one toxic sample of 192 examined. If one examines the data from Table 3 covering combined addition of lactobacilli and sucrose, without considering nitrite level, one finds just 2 toxic samples out of 271 tested.

Application of lactobacilli directly to the finished product, as was done here, may be regarded as a cumbersome method of distributing culture when compared to incorporation in the pickle solution. It may be necessary to do it this way in certain manufacturing procedures, however, because of the wide variation in smoking and heating times and temperatures employed. There are probably manufacturing processes which would inactivate or drastically reduce the viability of the culture if it were added with the pickle.

The two conditions that must exist for success by the sucrose-lactic organism system are an adequate amount of fermentable carbohydrate and an adequate bacterial inoculum. When these conditions are met, the level of nitrite can be reduced to whatever quantity is required to make an organoleptically acceptable product. Since this should be well below the currently required 120 ppm, lower residual nitrite levels and less nitrosamine formation can be reasonably expected.

The organism used in this study, *L. plantarum*, showed little growth, but good survival through 21 days at 4 C. This then provides a system in which properly refrigerated bacon is not subject to acid flavor, but the organism is available for acid production should the product undergo temperature abuse.

While sorbate was not given important consideration in this study, the limited data show some inhibitory effect on botulinal toxin formation, though this was overcome after a few weeks of temperature abuse when no sugar

TABLE 1. *Residual nitrite detected in incubated samples.*[a]

		Added				$NaNO_2$ detected (ppm) at incubation days at 27 C					
Experiment	Batch	$NaNO_2$ (ppm)	Sucrose (%)	*L. plantarum*	Other	0	2	3	4	5	7
1[b]	A	80	0.9	–[c]	–	14	NA[d]	NA	NA	NA	NA
	B	120	0.9	–	–	34	NA	NA	NA	NA	NA
2	D	120	0	–	–	40	25	NA	8	NA	3
	E	120	0	+[e]	–	40	5	NA	3	NA	NA
	F	120	0.9	–	–	30	16	NA	4	NA	NA
	G	120	0.9	+	–	30	4	NA	3	NA	NA
3[f]	D	120	0	–	–	18	8	NA	NA	NA	NA
	E	120	0	+	–	4	3	NA	NA	NA	NA
	F	120	0.9	–	–	9	4	NA	NA	NA	NA
	G	120	0.9	+	–	4	4	NA	NA	NA	NA
					0.31% STPP						
4	A	120	0.9	+	+	34	NA	3	NA	NA	NA
	B	120	0.5	+	+	37	NA	3	NA	NA	NA
	C	120	0.5	+	–	24	NA	3	NA	NA	
	D	120	0.1	+	–	20	NA	2	NA	NA	NA
					0.26% K-Sorbate						
5	A	40	0	–	–	17	NA	13	NA	9	5
	B	40	0	–	+	11	NA	7	NA	6	5
	C	40	0.9	+	–	13	NA	10	NA	4	NA
	D	120	0	–	–	33	NA	24	NA	21	8
	E	120	0	–	+	41	NA	33	NA	22	9
	F	120	0.9	+	–	37	NA	8	NA	5	NA

[a]Sodium nitrite was assayed in bacon which was not inoculated with *C. botulinum* spores but otherwise treated in the same way as the inoculated samples.
[b]The numbers correspond to the numbers of figures (Experiment 1 shows the results obtained in the experiment reported in Fig. 1, etc.).
[c]Not added.
[d]Not assayed.
[e]Added.
[f]These samples had been stored for 21 days at 4 C, then inoculated at 27 C. During the refrigerated storage period, residual nitrite values assayed were as follows:

	$NaNO_2$ detected (ppm) at storage days at 4 C			
Batch	0	7	14	21
D	40	20	16	18
E	40	24	1	4
F	30	17	4	9
G	30	17	1	4

TABLE 2. *Analytical data on 0-time samples.*

Experiment	Batch	*C. botulinum* (MPN/g)	NaCl (%)	Moisture (%)	Aerobic count[a] per g
1[b]	A	1.0×10^4	—[c]	—	—
	B	1.6×10^4	—	—	—
2	A	1.9×10^3	—	—	8.2×10^6
	B	1.6×10^3	—	—	$< 2 \times 10^3$
	C	1.5×10^3	—	—	7.4×10^6
	D	2.0×10^3	—	—	$< 2 \times 10^3$
	E	1.5×10^3	—	—	7.4×10^6
	F	1.3×10^3	—	—	$< 2 \times 10^3$
	G	1.8×10^3	—	—	1.2×10^7
4	A	3.4×10^3	1.50	39.6	—
	B	2.3×10^3	1.49	39.8	—
	C	2.7×10^3	1.45	46.0	—
	D	1.7×10^3	1.58	49.1	—
5	A	3.1×10^3	1.38	33.0	—
	B	3.9×10^3	1.57	39.4	—
	C	4.0×10^3	1.82	43.5	—
	D	2.4×10^3	1.36	38.0	—
	E	4.4×10^3	1.67	40.0	—
	F	2.7×10^3	1.54	32.2	—

[a]Aerobic count was done on APT agar plates.
[b]The numbers correspond to the numbers of figures (Expt. 1 = Experiment reported in Fig. 1, etc.;
[c]Not done.

TABLE 3. *Summary of toxicity results.*

Variables			Results of toxin assays	
$NaNO_2$ (ppm)	Sucrose[a] added	Lactobacilli added	Total number of samples tested	Number of toxic samples
0	—	+	27	26
0	+	—	52	50
0	+	+	49	1
40	—	—	50	47
40	+	+	30	0
120	—	—	28	17
120	—	+	68	34
120	+	—	149	4
120	+	+	192	1

[a]Sucrose concentrations were 0.5% or higher.

was added to the system.

Ultimate refinement of this process requires that work be done to answer several questions. Are there better species and strains of lactic acid bacteria for optimum growth and acid production under abuse conditions without creating a problem of unwanted acidity in refrigerated storage of the product? What strains are most tolerant to bacon processing conditions, and will survive best at storage temperatures? What is the minimum required concentration of fermentable carbohydrate? What are the advantages of adding culture to the pickle vis-a-vis direct application, either before or after slicing?

ACKNOWLEDGMENT

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Detection and Prevention of Post-Processing Container Handling Damage

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ABSTRACT

Specific recommendations for detection and prevention of post-processing container handling damage are best established through a detailed on-site analysis of each conveyor line. Procedures for making such an analysis are given as well as general handling recommendations for most container handling systems.

The canning industry's use of lightweight metal containers and faster container conveyor speeds has led to an increased effort to minimize container damage. The reluctance of consumers to purchase damaged containers and increased emphasis by regulatory agencies are also reasons for renewed efforts in preventing handling damage.

This paper is primarily concerned with physical damage to hermetically sealed rigid metal and glass containers after thermal processing. It describes the types of damage to look for and suggests a program for regular examination of container handling equipment. In addition, general recommendations for most container handling systems will be presented. A greater emphasis will be placed on these aspects for metal containers than glass containers whose fragile nature imposes somewhat self-limiting procedures for their handling.

The significance of container damage has been emphasized in numerous publications. Perhaps the biggest problem resulting from container damage is spoilage of the processed product due to contamination during the loss of container integrity. Smith (*7*) stated that the three main factors in leaker type spoilage in metal cans were (*a*) the condition of the double seams, (*b*) improper installation or operation of post-processing can handling equipment leading to can abuse and (*c*) the presence of microbial contamination in container cooling water. Demsey (*4*) used a "Bio-Test" procedure to relate can abuse by specific post-cooling can handling equipment to leaker spoilage. Through in-plant studies Braun and Pletcher (*3*) and Bohrer and Yesair (*2*) showed that, despite the use of chlorinated cooling water, leaker spoilage can result when rough can handling occurs on contaminated container handling tracks and equipment. In review, Put et al; (*6*) described the mechanism of microbial leakage into a metal can and the influence of seam construction and can handling procedures upon its occurrence. Numerous recommendations for reducing container handling damage and related spoilage have been presented by Bohrer (*1*), Troy and Folinazzo (*8*), Troy et al. (*9*) and N.C.A. (*5*).

BENEFITS

The greatest benefit that can be realized from use of a comprehensive program to prevent can damage is one of the economics. The reluctance of consumers to buy dented or otherwise damaged containers adversely affects repeat sales and brand name acceptance. The yield and profit of a plant can be significantly affected when containers have to be discarded due to damage before shipment.

It is obvious that spoilage in the warehouse due to damaged containers is a direct economic loss. It is not as well recognized that such spoilage often causes the loss of sound product due to contact with product from bursting or leaking containers. Labor savings may also be accomplished by reducing the need to rework cases that have some damaged containers in them.

Spoiled product in the warehouse provides an attraction to insects and rodents. This could lead to increased costs of an extermination program and findings by regulatory officials of adulterated product.

Considerable expense would be involved if it became necessary to recall spoiled product due to container damage problems discovered after shipment.

DESIGN OF A PROGRAM

Occasional spot checks for container damage are of value, but may not provide the detailed examination needed to uncover the full extent of damage. Establishment of a organized container damage program may be a worthwhile effort for those that find there is a high level of damage in their operations.

Before such a program is established, an initial survey should be made to determine the extent of production loss due to container damage. Such a survey may only involve a comparison of incoming container or production figures with warehouse inventory and shipping records or it may be desirable to add to this a more detailed examination at each stage of container handling. The result of this initial survey should provide evidence of whether or not an organized container damage program is needed.

If it is found that such a program is needed, its establishment should begin with a designation of management responsibility. The overall plan should involve training of plant operating personnel, coordination with maintenance and purchasing and a schedule for regular examinations for damage on each line.

Training of plant personnel to be aware and watchful for abnormal and damaged containers will provide an opportunity for constant inspection. The abnormal containers removed from the lines should be saved in designated receptacles. These containers should frequently be examined by designated management for extent, type and similarities of defects. Such a plan can provide an early indication of damage problems and where they are occurring. Plant personnel should also be made aware that when a large number of damaged containers are found, they should immediately notify a designated person. Such a plan could prove valuable in preventing serious problems from developing.

Coordination of the program with maintenance personnel will aid in locating problem damage. This will also be helpful in finding out what container handling equipment requires frequent adjustments and repairs. Additional information will be provided to determine the types of equipment that perform best on a particular line. This information should be coordinated with those responsible for purchasing container handling equipment to establish the best type of equipment for a particular system.

The program should be established with a schedule for regular inspection of handling problems on each container line. The frequency of such a schedule depends upon the speed of the line, the extent of damage found on the initial survey or the last inspection, and when changes or repairs were made on the handling equipment. Scheduled inspections will be helpful in keeping the program viable, but most of all it will provide a preventative feature instead of a reactive one.

WHAT TO LOOK FOR

A container damage inspection will be of greatest value if the person making the inspection knows what to look for and has a guide to aid in determining acceptable and unacceptable damage. Assistance in preparing such a guide can be obtained from the information and illustrations in the National Food Processors Association (N.F.P.A.) bulletin 38-L (2nd edition) entitled, *Guidelines for Evaluation and Disposition of Damaged Canned Food Containers.* This bulletin is available from N.F.P.A., 1133-20th Street, N.W., Washington, D.C. 20036. Two audio visual tape-slide presentations entitled, *Safe Can Handling for Cannery Employees* and *Can Handling,* are available from the Food Processors Institute, 1133-20th Street, N.W., Washington, D.C. 20036. Additional assistance could also be obtained from your container supplier.

SOME EXAMPLES OF UNACCEPTABLE DAMAGE

Empty metal containers and ends

1. Fractures in the metal plate or pinholes
2. Cable cuts or fractures through the metal
3. Sharp dents and deformed body flanges
4. Deformed or out of round cans
5. Skips in the compound of metal ends

Filled metal containers

1. Leaking containers
2. Stained labels
3. Severe body dents
4. Buckled or swollen ends
5. Fractures or dents on the score lines of easy open cans
6. Pitting and heavy rust
7. Die code fractures
8. Crushed cans
9. Moderate to severe dents at the side seam junctures
10. Cable cuts or fractures through the metal
11. Open cut-overs at the side seam juncture
12. False seams
13. Incomplete seaming operation
14. Knocked down flanges
15. External vees or droops

Empty glass containers and caps

1. Cracked or fractured body
2. Chips or fractures in the finish and sealing surface
3. Inadequate gasket material in caps

Filled glass containers

1. Raised or cocked caps
2. Loose caps
3. Cracked or fractured body
4. Leaking containers
5. Stained labels
6. Improper application of caps

SOME EXAMPLES OF ACCEPTABLE IMPERFECTIONS

Metal containers

1. Flat rim dents in double seam not at side seam junctures
2. Minor body dents
3. Minor external rusting
4. Slight to moderate dents on or near the double seam
5. Slight to moderate paneling on the sides

Glass containers

1. Minor dents in center panel of caps
2. Small imperfections on the finish

The examples listed above provide a general guide of acceptable and unacceptable container defects. It should be recognized that variations in any of these examples could change their assigned category.

CONDUCTING AN INSPECTION

An organized procedure is required to perform a successful container damage inspection. A common sense approach to the inspection is most useful. Many areas of can handling damage are associated with elevated noise levels resulting from the impact of metal against metal. In other places, simple visual observation can readily detect handling operations which need to be smoothed out.

Inspection forms will aid in making the survey and also in interpreting its results. These forms should be individually designed for each plant. They should be simple to complete, but contain adequate detail to make the collected information useful. At a minimum, the

forms should allow distinction between production lines, show where defects are uncovered and indicate the magnitude and type of damage.

It is logical to begin an inspection in the finished product warehouse. Problems uncovered here will show the cumulative effect of all damage encountered during production from beginning to end, except for any gross damage which resulted in removal and disposal of containers during production.

The first things to look for in the warehouse will be obvious damage, such as that caused by improper fork lift truck operation or other forms of mistreatment of the finished goods. Frequently, can damage is easily recognized by wet cases which result in leakage or bursting of containers of spoiled product.

Less severe damage present in the warehouse will be found only by opening cases and sampling the cans within. Information on the sample size needed to uncover significant damage is available from statistical probability tables. Once the appropriate sample size is determined, random samples should be obtained. Containers of each size and from each production line should be included.

The sample cans should then be visually examined very closely for defects. The type and degree of damage should be recorded on the inspection forms. Particular note should be made of repetitive types of damage. The absence of damage should also be recorded on the forms, and in that event, the need for further inspection would not be indicated. However, if extensive major or even minor damage is uncovered in the warehouse or if significant numbers of damaged containers are removed from the line during production, the investigation should continue until the source of the damage is located.

Often information from the warehouse inspection or from observation of production line rejects will suggest that a particular piece of equipment is the source of a specific type of damage. At other times, a more systematic approach is necessary.

In this situation, the investigation should proceed from the warehouse countercurrently to container flow along the production line. At each equipment discharge along the way, statistically sound numbers of samples should be taken and examined, with the results being recorded. The examination should continue upstream to a point where the type of damage being observed is no longer found or where there are no damaged cans in the sample. The source of the damage should be located between this point and the previously sampled point. Once the cause of the damage is discovered, appropriate steps can be taken to correct the problem.

GENERAL HANDLING RECOMMENDATIONS

Each container handling system is different in some aspects from another and no set of specific recommendations will be suitable in all instances. However, there are some general procedures that do apply to most handling systems for rigid containers.

Containers rolling down conveyors at high speed are particularly susceptible to damage. This damage usually occurs when the containers contact each other, especially if there is a sudden stop at the end of the line. Retarders made of non-porous material should be used to slow the speed of the containers and reduce impact. Keeping containers separated from each other on the conveyor line and adjustment of transfer points from one piece of equipment to another will also prevent impact damage.

Container elevators should be adjusted to take containers away faster than they are fed to it. This will reduce damage from container contact. These elevators should also be constructed with non-porous shock absorbers to prevent flat rim dents.

Lowerators, especially the zig-zag type, are often the cause of rim dents. The best way to prevent this is to lengthen the horizontal runs, increase the radius of the curves and thereby reduce the number of direction changes.

Container track adjustments and guide clearances should be as narrow as possible to prevent seam-to-body contact denting. The larger the radius of track curves, the easier this is to accomplish.

These recommendations do not include all types of handling equipment that may be encountered. Direct consultation with manufacturers of container conveying equipment will often be useful in solving problems of container damage.

SUMMARY

A systematic and organized container damage inspection program will be helpful in detecting rigid metal and glass container handling problems. The establishment of a guide of acceptable and unacceptable defects and the use of inspection forms to record the results will provide uniformity in the program. Coordination of such a program with the equipment maintenance and purchasing departments under management control will be valuable in preventing serious container damage problems. Training of all operating personnel to practice gentle container handling will be the most important factor in producing the greatest number of defect-free containers.

ACKNOWLEDGMENTS

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Prevention of Post-Process Contamination of Semi-Rigid and Flexible Containers

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ABSTRACT

Leakage of semi-rigid and flexible containers can be caused by faulty sealing or pinholes. Semi-rigid containers can be closed satisfactorily by removing product from the seal area during sealing. Biotesting indicates that pouches can be even more leakage-resistant than cans, provided the seal area is kept clean by proper filling. Careful handling keeps pinholes in these packs to an acceptably low level. Pouches experimentally punctured after processing result in low contamination provided they are immediately dried after cooling in chlorinated water. Large-scale production over several years of flexible pouches in Japan and semi-rigid containers in Europe has resulted in very little post-processing contamination.

Heat-processed canned foods have been on the market for more than 100 years. Several years ago, advanced types of packaging appeared on the market. In Europe, mainly semi-rigid containers are used. Japan, however, produces about 500 million flexible pouches annually. Both types of packaging have much in common in that they consist of an aluminium laminate with a heat-sealable compound on the inside. There are also many differences.

(*a*) Semi-rigid containers are made by deep-drawing reel material. This material consists of a laminate of aluminium (thickness about 100 μm) with a polyolefin layer on the inside and a lacquer layer on the outside. The aluminium laminate is sometimes coated with a heat-sealable, retortable layer of lacquer based on epoxy resin. The aluminium layer gives the container its mechanical strength and protects the contents of the container against the influence of light and oxidation by oxygen. The polyolefin layer protects the aluminium layer against corrosion and makes heat-sealing possible. The lid of the container is, in most instances, made of thinner film and heat-sealed to the container after filling (Fig. 1, 2).

(*b*) Flexible pouches are also made from aluminium laminate (Fig. 3, 4). Here, the aluminium layer thickness should at least be 8 μm to avoid pinholing. Also in this instance, does the aluminium layer protect the product against light and oxygen. The outside of the aluminium film is coated with polyester and the inside with a heat-sealable polyolefin layer, which also adds to the strength of the material. Generally, three sides of the pouch are already sealed by manufacturers, while the fourth is sealed after filling.

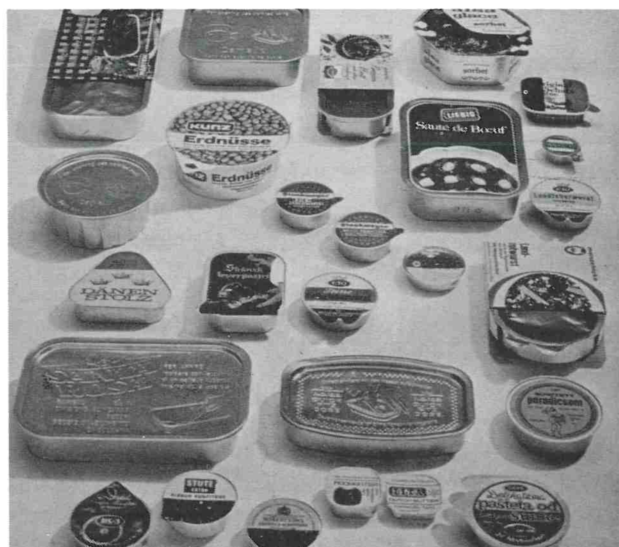

Figure 1. *Semi-rigid containers.*

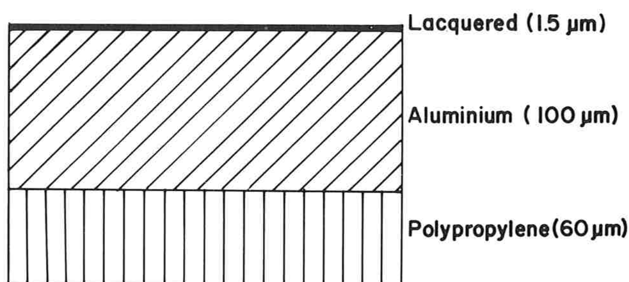

Figure 2. *Cross-section of a laminate used for semi-rigid containers.*

SEALING

Both types of packaging are closed by heat-sealing. The polyolefin layers are fused under "high" pressure and temperature, and to ensure good sealing both surfaces should be plane-parallel at the moment of sealing. Besides, the jaws of the sealing equipment should be exactly parallel and the temperature and pressure should be equal over the whole sealing area. With semi-rigid containers, small distortions in the rigid aluminium are flattened out under high pressure but with flexible containers the sealing area should be stretched to avoid that wrinkles are pressed into the rims to be sealed.

Semi-rigid containers are closed horizontally, the sealing width varying between 1.5 and 4 mm. The upper

Figure 3. *Flexible pouches.*

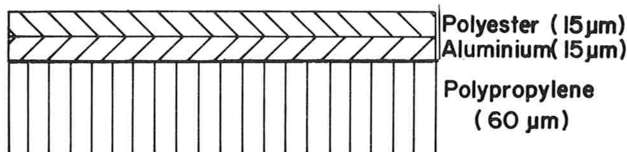

Figure 4. *Cross-section of a laminate for flexible pouches.*

jaw of the equipment has a temperature of about 250-300 C and the lower one should be below 100 C. Due to the high pressure (about 50-200 kg/cm^2), it is possible to squeeze product away from the sealing area. The seals are so designed that the product is always moved downwards. As a result only the lower sealing jar may be contaminated with product. To avoid burning problems, the temperature of the lower jaw should therefore always be below 100 C.

Flexible packages are closed vertically and their seals are much wider (sometimes 1 cm). The jaw temperature is about 150-170 C and the sealing pressure about 1.2 kg/cm^2. When a higher pressure would be applied, the aluminium layer might be damaged. Particularly for this type of packaging, it is important that the sealing area is absolutely free from product. Otherwise, proper sealing will be impossible, because the sealing pressure applied is too low.

QUALITY CONTROL OF SEALING

Cans are checked visually (seam damage, seam thickness, overlap, etc.) but a systematic inspection of seals is not so easy. The following parameters may be used to check seal integrity.

Bursting pressure

Generally, the bursting pressure is used as a tool to check seal integrity. The pressure to be applied depends on the type of material and need therefore be specified by manufacturers.

Measurement of thickness

The seal thickness of semi-rigid containers can be measured. Due to the high pressures exercised, the aluminium becomes perfectly flat. The combination of high temperature and pressure should produce complete fusing of the two polyolefin layers of lid and container. The seal thickness is therefore an indication of the quality of the seals. For flexible pouches, this thickness measurement provides no such indication of seal quality.

Creeping liquid

To detect a place in the seal not properly sealed, for instance, as a result of contamination with product, the following method may be used. The seal is cut from the packs after which the aluminium layer can be removed by etching. Into the inside of the remaining clear plastic seal, a colored creeping liquid is introduced so that, after several minutes, the place not properly sealed will show up.

Biotest

The main reason why packs should be closed and remain closed during handling and distribution is to prevent microorganisms from entering the packs. A reliable method to determine the amount of leakers in a large batch is a biotest. We developed one similar to the biotest applied by Put et al. (*3*). The microorganism used is the same, but a more nutritious medium is used in our method. It also contains a pH indicator to check if the organism grew. Similarities and differences between semi-rigid and flexible containers are summarized in Tables 1 and 2.

MICROBIOLOGICAL ASPECTS OF SEMI-RIGID CONTAINERS AND FLEXIBLE POUCHES

With the aid of a biotest, we determined the rate of leakage of various types of semi-rigid containers. The results are given in Table 3. It can be concluded that it is possible to close various types of semi-rigid containers satisfactorily. Considering the thickness of the aluminium layer, it is unlikely that pinholing will occur; however, this possibility cannot be excluded in relation to the more vulnerable flexible containers. Since it is very likely that pinholing will increase during processing, we handled a group of pouches extremely roughly and another group very carefully. Both batches were

TABLE 1. *Aspects of semi-rigid and flexible containers.*

Aspect	Semi-rigid containers	Flexible containers
Sealing pressure	high	low
Plane parallel	pressing	stretching
Surface	preferably clean	absolutely clean

TABLE 2. *Quality control of semi-rigid and flexible containers.*

Method		Semi-rigids	Flexibles
Bursting pressure	determination of strength of seal	+	+
Thickness	plane parallel check on heat input	+	–
Creeping liquid	detection of seal defects	+	+
Biotest	detection of defection levels	+	+

TABLE 3. *Rate of leakage of various types of containers.*

Type of container	Number of investigated containers	Percentage of leaking containers
Tin can	1000	0.0
Semi-rigid container of aluminium-polyethylene (125 ml)	610	0.3
Semi-rigid container of aluminium-polypropylene (130 ml)	500	0.8
Semi-rigid containers of lacquered aluminium (50 ml)	1000	0.3

subsequently biotested. The results (Table 4) clearly show that the real seal defects hardly ever occurred with proper sealing conditions, but that an appreciable number of leaking pouches was found under conditions of rough handling.

When using a biotest to determine quantitatively the amount of leakers, the concentration of microorganisms should be sufficiently high to guarantee that all leakers are infected. Usually $> 10^8$/ml is necessary to obtain acceptable results. When the concentration of microorganisms is lowered, fewer leakers will be detected, which has already been shown by Put et al. (*3*). We did a similar experiment with flexible pouches. They were pierced on both sides of the pack with a needle of 100 μm. After processing, these packs were kept for 30 min in a biotest suspension containing different concentrations of microorganisms. After this treatment, the packs were washed and dried carefully and incubated for 1 week at 30 C. The results (Table 5) show that when using a low concentration of microorganisms only few defective pouches were detected.

TABLE 4. *Effect of handling of pouches on the number of pinholes.*

Handling of pouches	Number of of pouches	Number of seal defects	Number of leaks through pinholes	Percentage
Rough	5616	2	18	0.32
Careful	3744	3	2	0.08

TABLE 5. *Contamination of pouches vs. number of* E. aerogenes *cells in the biotest suspension.*

Number of microorganisms per ml	Percentage of contamination
130	18
20 000	52
520 000	100
130 000 000	100
infection via cooling water	4.2

In the previous experiment, we used a contact time of 30 min and it may be expected that at a shorter contact time, the number of infected pouches will be lower. We therefore carried out a series of experiments with pouches with a hole of 100 μm on each side. Each series of 100 pouches was introduced into a bacterial suspension of 10^4/ml for a certain period. The results are given in Table 6. These experiments show that, provided the contact time is very short, only some leaking pouches become contaminated when contacted with a small number of microorganisms.

TABLE 6. *Degree of contamination of pierced pouches (two 100-μm holes) at various contact times.*

Contact time (min)	Organisms per ml	Percentage of contamination
1	1.5×10^4	15
5	4.0×10^4	58
15	4.6×10^4	57
30	2.3×10^4	45

PREVENTION OF POST-PROCESS CONTAMINATION

The level of microorganisms in cooling water should be kept as low as possible (*2*). Diminishing the contact time has also been suggested as a means of preventing post-process contamination (*4*). Against this background, we did a series of experiments to establish whether this can be achieved under practical conditions. Since the results of these experiments have already been published (*1*), they will be dealt with briefly.

Pouches were perforated on both sides with a needle of 100 μm. After the heat treatment, they were treated in three different ways. One series was roughly handled by hand and stored under wet conditions at 30 C for 1 week. A second series was dried immediately after cooling and packed in protective cartons and stored at 30 C for 1 week. A third series was handled under wet conditions, manually dried, packed in cartons and stored at 30 C for 1 week.

The results show a very high degree of infection for the wet-stored packs and a low infection rate for the dry-stored packs. This means that even for pouches with two holes of 100 μm each, the rate of post-process contamination will remain low, provided the pouches are dried quickly. It may therefore be concluded that well-closed packages, which are cooled in chlorinated cooling water, dried quickly after cooling and then packed in cartons, will have an acceptable safety margin.

Interesting is that not all contaminated pouches showed bombage after incubation; about half of these packages showed no bombage at all.

We also studied the effect of transportation on post-process contamination. For this purpose, we pierced 400 pouches. After cooling and drying, we packed them in cartons which were transported throughout Holland for 1 week. After incubating them for 3 weeks at 30 C, we found that only 2% was contaminated. With the same batch of pouches, we transported 2000 non-leaking pouches which were biotested after the transport test. It appeared that none of these showed any leakage.

CONCLUSIONS

Our experiments allow of the following conclusions: (*a*) in a biotest, semi-rigid containers and flexible pouches show a low percentage of leakage; (*b*) careful control during sealing will keep the number of defective seals low; (*c*) post-process contamination can be kept to a low level when working under hygienic conditions, especially when quick drying on the containers after cooling is applied; in this way, it is possible to reduce post-process contamination to an acceptable low level; and (*d*) during

transport, the number of contaminated packs does not increase if they are properly cartoned. We therefore conclude that it is possible to produce sterilized products packed in semi-rigid containers or flexible pouches which will reach the consumer in excellent condition.

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damaged the surfaces tested.

A second method involved rolling a small, plastic hand roller across the polyethylene film on the soiled surface. This method was undesirable as the force applied to the polyethylene film varied from sample to sample because the operator of the roller had to apply pressure, which could not be accurately duplicated, to effect transfer of lipid to the polyethylene film from the food contact surface. This method eventually led to the method used in all further work which was to use a relatively heavy roller and apply no pressure to it while taking the samples.

One other method of artificially soiling the food contact surfaces was also investigated. This method involved dissolving a known amount of lard in a known volume of petroleum ether. Open-ended plexiglass cylinders approximately 6 cm high were placed on the food contact surface. One mililitre of the lard-ether solution was placed on the food contact surface within the center of the cylinders. The ether was allowed to evaporate and the cylinders were removed, leaving a film of lipid on the food contact surface. One problem with this method was that the lipid was not deposited evenly over the food contact surface. A second problem was that the lard-ether solution was not totally contained within the cylinders, thus the exact area soiled was not known. Applying vacuum grease or vaseline to the edges of the cylinder was not acceptable since residues of these materials were extremely difficult to remove from the food contact surfaces, and could have had an influence on the properties of the food contact surface.

With further development, the method described in this paper may be well suited for use in routine quality control measurements of lipid residues on previously cleaned equipment in food handling plants. It is quite sensitive to the presence of small amounts of lipid especially on HDPE, stainless steel and glass. This method is also rapid, safe and simple to perform and samples can be easily obtained in the field with a minimum of equipment.

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Food Poisoning Associated with Post-Process Leakage (PPL) in Canned Foods

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ABSTRACT

One hundred and fifty-four incidents of food poisoning were associated with post-process leakage (PPL) between 1921 and 1979. These occurred mainly in Great Britian (72.7%) and Canada (17.5%) from products exported from South America, Europe, Africa and Australia. Defects leading to leakage were identified as defective seams and perforations during processing; temporary microleaks during cooling; and case-cutter damage, punctures, corrosion and dents after processing. Organisms associated with the incidents were *Staphylococcus aureus* (100, 64.9%), *Salmonella typhi* (6, 3.9%), other *Salmonella* spp. (9, 5.8%), *Clostridium botulinum* (3, 2.0%), *Clostridium perfringens* (3, 2.0%), others and undetermined 33 (21.4%). Canned meat, fish and vegetable products were involved. In particular, corned beef contaminated with *Staphylococcus* or *Salmonella* caused 53 incidents; pork and ham products contaminated with the same organisms caused 16 incidents. Where information was available, it was found that the median amount of meat contaminated with *Salmonella* consumed by ill persons was 105 g. For *Salmonella*-contaminated fish the amount associated with illness was between 40 and 320 g. Although many of the PPL incidents recorded occurred decades ago, significant outbreaks from this cause have appeared in the last few years. Appropriate action should be taken to reduce PPL at the manufacturing and retail level.

Canned foods are among the safest of food available to the public today. The safety of canned low-acid foods is due to the heat processes that they must undergo to prevent outgrowth of *Clostridium botulinum* spores and the even more severe heat treatment to prevent spoilage; the safety of high-acid foods is primarily due to their low pH. In addition, all stages of the manufacture of canned foods should be carefully controlled to prevent recontamination of the products by providing sound cans, satisfactory seaming operations, safe cooling water and careful sanitary handling after processing. This was not always so; for example, in Britain from 1914-1923, 30 of 86 reported food poisoning outbreaks (36%) were due to canned food, including beef, salmon, mutton, sardines, tongue, crustacea and fruits (*75*). The high association of canned food and illness was considered a "fair estimate" because a "considerable prejudice" existed against canned foods at the time, and outbreaks implicating them were brought to the "notice of the authorities more readily than with another vehicle" (*73,75*).

In the first quarter of the 20th century, the known etiological agents of foodborne disease from canned foods were *Salmonella* spp. and *C. botulinum; Staphylococcus aureus* and *Clostridium perfringens* were not known to be capable of causing food poisoning, although staphylococci were sometimes found in canned foods implicated in outbreaks . Savage and White (*75*) were so convinced of the prevalence of salmonellae in food poisoning from canned food that they attributed some outbreaks to *Salmonella* "toxins" even when viable organisms could not be found. Our retrospective evaluation of the symptoms described in their report indicate strongly that foodborne staphylococcal intoxication had occurred. Food poisoning from *S. aureus* was not generally recognized until the 1930s (*2*), and from *C. perfringens* until the 1940s (*59*). In this review, we present a survey of food poisoning that resulted from post-process leakage (PPL) of canned foods. The basis for the survey are reports published between 1925 and 1979 and unpublished information.

CRITERIA FOR FOOD POISONING RESULTING FROM PPL

Evidence for food poisoning from PPL usually requires that the first three conditions presented in Table 1 be met. Canned foods that caused food poisoning are obviously not spoiled to the extent that they would be rejected, although in some incidents such food has been reported to have tasted, smelled or looked abnormal. Frequently, neither the food nor the can is available by the time a food poisoning outbreak is recognized, and incrimination of the food is based only on epidemiological evidence (*11*). Once a canned food has been identified as the cause of an outbreak, the post-processing nature of the contamination may be established by identifying the defect in the can, if available, or in other cans by the same food processor, although PPL can occur without any obvious defect in the container (microleaks, or "breathing" according to Segner (*76*). To establish food poisoning from PPL, under-processing, scombroid poisoning, and heavy metal and other chemical

TABLE 1. *Criteria to determine whether food poisoning has resulted from post-process leakage (PPL).*

1. Food not spoiled sufficiently to prevent consumption
2. Illness epidemiologically associated with canned food eaten
3. Can freshly opened *or* evidence for post-process contamination *or* can defect identified *or* under-processing ruled out
4. Organism or toxin may be found in empty can *or* food *or* patient
5. Other cans of food from same factory may be found to be contaminated with same organism as that causing illness *or* other heat-sensitive organisms

poisoning should be ruled out, while the presence of heat-sensitive organisms, particularly staphylococci, salmonellae and *C. botulinum* type E, in freshly opened cans provides good evidence.

TYPES OF CAN DEFECTS ASSOCIATED WITH PPL FOOD POISONING

The types of defects that occur in cans and may lead to PPL are listed in Table 2. Although some of these were probably more prevalent in the early days of canning, all of them continue to cause problems and have led to illness in the last few years. PPL is of two main kinds, one in which organisms are drawn into the can by negative pressure during the cooling process, and the other in which organisms penetrate through holes originating during post-process handling and distribution of the product to the retail store or to the home. The canning industry is more aware of the former than the latter kind, since after-distribution damage resulting in a PPL food poisoning usually affects only one or two cans in a lot.

Table 3 shows details of the various kinds of after-processing damage that can lead to illness. Case-cutter damage occurs when employees at a retail store cut through cardboard boxes to expose the packed cans. Often a knife will cut the label and scratch the surface; occasionally the knife may penetrate the container. Organisms may be carried in with the tip of the blade. If the can contents are to any extent viscous the hole may seal with no apparent leakage. Other forms of puncturing may occur; a tool is used accidentally or deliberately to make a hole in a can, or the code is embossed too heavily into the can end, causing the metal to be very thin and perforated. The contamination of canned salmon with *C. botulinum* type E may have come through a cut, possibly caused by a saw (*14*), or conveying equipment (*13*). Corrosion can take place if the can has been unused for several years or is kept under wet conditions. Dents may be responsible for springing leaks in cans, as the one described by Bowmer et al. (*26*).

TABLE 2. *Types of can defects associated with PPL[a].*

A. During processing, especially cooling
  1. Temporary microleaks (breathing) in normal cans
  2. Cracks and defective seams
  3. Abrasions
  4. Perforation in lid due to embossing
B. After processing, particularly after distribution
  1. Case-cutter damage
  2. Punctures
  3. Corrosion
  4. Dents

[a]Categories A and B are not mutually exclusive, e.g., temporary microleaks may occur as a result of heating during a fire or from rough handling.

TYPES OF ETIOLOGICAL AGENTS ASSOCIATED WITH PPL FOOD POISONING

The types of organisms associated with illness resulting from PPL are listed in Table 4. *S. aureus* was responsible for the largest number of incidents (100 of 154, 64.9%); most of these were small in size. Outbreaks caused by *Salmonella* spp. and *C. botulinum* type E were considerably fewer, but were more serious in nature, since severe illnesses and deaths occurred. Agents not well documented as causing PPL, e.g., *C. perfringens*, fecal streptococci and molds, are discussed in the text.

TABLE 4. *Etiological agents associated with PPL food poisoning.*

Agents	Incidents: Number	Incidents: Percentage
Staphylococcus aureus	100	64.9
Salmonella typhi	6	3.9
Other *Salmonella* spp.	9	5.8
Clostridium botulinum	3	2.0
Clostridium perfringens	3	2.0
Coliforms, streptococci, clostridia and/or micrococci	4	2.6
Suspect mold	6	3.9
Undetermined	23	14.9
Total	154	100.0

TABLE 3. *Illness from PPL that occurred after processing.*

Type of defect	Canned food	Etiology	Number of cases	Year	Country where food eaten	References
Case-cutter damage	pea soup	suspect mold	1	1974	Canada	(*20*)
Case-cutter damage	vegetable soup	?	1	1974	Canada	(*20*)
Case-cutter damage	tomato soup	?	1	1975	Canada	(*21*)
Case-cutter damage	tomato juice	?	1	1976	Canada	(*77*)
Case-cutter damage	cream of chicken soup	suspect mold	1	1976	Canada	(*77*)
Case-cutter damage	baked beans	suspect mold	1	1976	Canada	(*77*)
Case-cutter damage	salmon	?	2	1976	Canada	(*77*)
Case-cutter damage	peas	?	1	1976	Canada	(*77*)
Case-cutter damage	cream style corn	?	1	1976	Canada	(*77*)
Puncture by screwdriver?	ham	*S. aureus*	3	1972	Canada	(*78*)
Puncture by nail	shrimp	*S. aureus*	2	1972	Canada	(*78*)
Puncture (unspecified)	tomato juice	suspect mold	2	1973	Canada	(*10*)
Puncture (unspecified)	macaroni and cheese	?	1	1975	Canada	(*21*)
Puncture of unknown origin	salmon	*C. botulinum* type E	4	1978	England	(*45*)
Corrosion	evaporated milk	*Streptococcus faecalis*	74	Mid 1940s	United States	(*30*)
Pin-hole leaks with corrosion	jugged hare	streptococci and micrococci	1	1974	Canada	(*20*)
Leaks in can possibly caused by dents	peas and carrots	*S. aureus*	20	1973	Canada	(*26*)
Total	17 incidents		117	1940s-1978	(3 countries, Canada, United States, England)	

?: information not available

STAPHYLOCOCCAL INTOXICATIONS ARISING FROM PPL [1942-1979]

We have found records of 100 incidents with well over 433 cases associated with canned meat, fish and vegetables (Table 5). Canned meat, particularly corned beef, was responsible for most of these. Eighty-three of the 100 outbreaks occurred in England or Wales, seven in the U.S.S.R., seven in Canada, and three in East Germany. Countries manufacturing the incriminated products were more widespread. Corned beef was imported from Africa, South America and Australia and pork products from Europe. In most incidents, single cans were involved, but the sizes of the cans are not mentioned. In some incidents, additional contaminated cans from the same lot were found and these yielded strains of *S. aureus* with the same phage type, or other identifying characteristics, as those which caused illness. The frequent mention of "no evidence of spoilage" and the discovery of additional unopened contaminated cans on investigation, indicate that many more persons could have eaten toxin-contaminated food. Thus, the 100 reported incidents are probably a small fraction of those that occurred.

Corned beef was the vehicle in 46 staphylococcal food poisoning incidents which occurred in England or Wales between 1942 and 1979; at least 235 persons were ill. In one of these outbreaks (*37*) the implicated can had a badly soldered seam, which allowed a leak to take place, and the same phage type of *S. aureus* was isolated from the corned beef, the food fragments in the can, and from

TABLE 5. *Staphylococcal intoxications arising from PPL.*

Canned food	Year	No. of outbreaks	No. of cases	Country where food eaten	Country of origin of product	Type of can defect	References
Corned beef	1942	1	> 14	England	?		(*4*)
	1954	2	13	England or Wales	Australia		(*34*)
	1954	1	4	England or Wales	Tanganyika		(*34*)
	1956	2	9	England or Wales	Tanganyika		(*34*)
	1957	1	?	England or Wales	Tanganyika		(*36*)
	1958	1	23	England or Wales	Argentina	badly soldered seam	(*37*)
	1958	3	10	England or Wales	Tanganyika		(*37*)
	1960	4	17	England or Wales	Tanganyika		(*38*)
	1960	4	10	England or Wales	Kenya or Tanganyika		(*38*)
	1960	5	14	England or Wales	Kenya		(*38*)
	1960	2	30	England or Wales	?		(*38*)
	1961	2	6	England or Wales	?		(*88*)
	1961	1	2	England or Wales	Tanganyika		(*88*)
	1962	2	6	England or Wales	Tanganyika		(*89*)
	1964	1	? (1 family)	England or Wales	South Africa		(*90*)
	1965	1	? (1 family)	England or Wales	Tanganyika		(*91*)
	1968	3	?	England or Wales	?		(*92*)
	1978	1	> 12	England	Argentina		(*44*)
	1979	7	> 61	England or Wales	Brazil		(*47,48,49,50*)
	1979	1	3	Wales	Malta		(*51*)
	1979	1	1	Canada	Australia	poor seams	(*77*)
Subtotal	1942-1979	46	> 235	England, Wales and Canada	6 countries (Africa, South America, Australia, Europe)		
Pork Products							
luncheon meat	1943	1	9	Wales	?		(*66*)
pork	1949-1952	2	15	England	imported, country unknown		(*71*)
pork	1950	1	?	England or Wales	Denmark		(*33*)
luncheon meat	1950	1	?	England or Wales	Czechoslovakia		(*33*)
pork brawn	1954	1	3	England or Wales	Denmark		(*34*)
chopped pork	1960	1	4	England or Wales	Poland		(*38*)
pork	1961	1	2	England or Wales	Germany		(*88*)
chopped pork	1974	1	4	England	Poland		(*42*)
chopped pork	1976	1	2	England	Poland	overlap of double seam defective	(*43*)
Subtotal	1943-1976	10	> 39	England and Wales	4 countries (Europe)		
Ham	1942	1	9	England	United States		(*3*)
	1960	1	12	England or Wales	Germany		(*38*)
	1972	1	3	Canada	Canada	punctures in lid	(*78*)
Subtotal	1942-1972	3	24	England, Wales, and Canada	3 countries (North America and Europe)		
Ox tongue	1950	1	?	England or Wales	Brazil		(*33*)
	1950	1	?	England or Wales	Denmark		(*33*)
	1954	1	5	England or Wales	Ireland		(*34*)
	1956	1	5	England or Wales	Denmark		(*35*)
	1965	1	?	England or Wales	?		(*91*)
Subtotal	1950-1965	5	> 10	England or Wales	3 countries (Europe, South America)		

Veal	1949	1	6	England	imported		(*71*)
Chicken	1960	1	3	England or Wales	United States		(*38*)
Fish and Shellfish							
Sprats in oil and smoked cod	1943-1947	7	?	U.S.S.R.	U.S.S.R.		(*81*)
kippered herring in oil	1956	3	7	East Germany	East Germany		(*69*)
shrimp	1972	1	2	Canada	United States	puncture	(*78*)
salmon	1973	1	5	England	?	seam leakage from faulty crimping	(*39,40*)
salmon	1978	1	2	Canada	Canada	microleak suspect	(*54*)
chicken haddies (hake, cod or cusk)	1978-1979	2	4	Canada	Canada	loose seams	(*29,77*)
Subtotal	1943-1979	15	> 20	4 countries (Europe, North) America)	4 countries (Europe, North America)		
Rice pudding	1973	1	3	England	?	crack in seam from dent	(*8,41*)
Peas and carrots	1973	1	20	Canada		seam leaks from dents?	(*26*)
Peas	1957	17	> 73	England	England	seam defects and microleaks	(*5,6,7,24,25, 79,80,85,86*)
Total	1942-1979	100	> 433	5 countries (Europe, North America)	16 countries (Europe, North America, South America, Africa, Australia)		

the patients.

Ten incidents (> 39 cases) were reported in England and Wales between 1943 and 1976 in which PPL of canned luncheon meat, chopped pork, pork brawn or unspecified pork products were implicated. Where known, these were all manufactured in European countries. In one incident, imported freshly opened canned chopped pork caused hospitalization of two people. Small amounts of enterotoxin A were found in the left-over refrigerated product and *S. aureus* strains isolated from food and patients produced enterotoxins A and C. Also, micrococci and *S. aureus* were isolated from additional cans of the same lot. The overlap of the double seam at the can maker's end was unsatisfactory. The importers agreed to withdraw the products of the factory concerned and not to import any more until the problem was corrected (*43*). Three incidents involving ham occurred in England or Wales and in Canada. Illness in the Canadian outbreak was caused through puncturing of the can by a tool, possibly a screwdriver (*78*).

Contaminated canned ox tongue was responsible for five incidents in Great Britain between 1950 and 1965. Unfortunately, in only two of these was the number of cases reported. The products came from Denmark, Ireland and Brazil. Canned veal and chicken each caused one episode to complete the total of 62 *S. aureus*-associated canned meat outbreaks.

Fifteen incidents associated with canned fish and shellfish products were reported between 1943 and 1979; there were more than 20 cases. The fish products came from eastern Europe or Canada. Three cans of the German kippered herrings, which had each caused an outbreak in 1956, were all produced in the same factory on the same day. The patients said that the fish smelled and tasted normal, although *S. aureus* was cultured from each can. Additionally, 3 of 39 unopened cans contained *S. aureus*. Isolates from all six cans and from many of the processing staff were of the same phage type. Another 22 of the 39 cans were contaminated with bacteria other than *S. aureus*. In another episode, salmon eaten in England contained 4×10^5 *S. aureus*/g and 1.2 μg of enterotoxin A/100 g. The route of entry of the organism was through faulty crimping of a seam. Entry of *S. aureus* via microleaks probably occurred in a can of Canadian salmon which caused two persons in Canada to be ill in 1978. Enterotoxins A (0.59 μg/100 g) and B (1.47 μg/100 g) found in the fish were in sufficient quantity to cause intoxication. No apparent holes were detected in the empty can, although the contents contained 1×10^3 *S. aureus*/g. The recent outbreaks from chicken haddies in Canada were attributed to staphylococcal contamination through improperly seamed cans.

The only vegetable products reported to have caused staphylococcal intoxication through PPL are rice, peas and carrots. Three people became ill after eating freshly opened canned rice pudding in England. *S. aureus* was isolated from the food (2.0×10^7/g), and a crack in the double seam at the site of a dent at the can manufacturer's end was the probable entrance for the organism. No other cans were found contaminated.

In a food poisoning outbreak in Canada in 1973, *S. aureus*, producing enterotoxins B and D, contaminated canned peas and carrots in a No. 10 can and caused 20 of 29 people at risk to become ill (*26*). Leaks were found in the can, possibly as a result of dents "on the bottom seam." The can was not likely to have been contaminated during processing because it was 6 years old when the contents were consumed. Since the phage and toxin types found in the vegetables and a food handler were identical, and the can was opened 1 h before the food was cooked, it was postulated that the food handler contaminated the can through leaks some days earlier.

In England in 1957, there were at least 17 outbreaks caused by canned peas, mostly, if not all, due to PPL. Eleven of the 17 were associated with consumption of peas that appeared normal and were from freshly-opened A10 (2.7 kg) cans manufactured at the same cannery, while two were apparently caused by A10 cans produced in another cannery. Bashford et al. (*24*) described two of these 17 outbreaks, both taking place at institutions (a school and a factory); 71 people became ill with typical symptoms of enterotoxin food poisoning. *S. aureus* phage type 42E was isolated from the implicated food, the cans and the patients' feces. In nine outbreaks (including the two above) phage type 42E (Group III) was isolated from victims, peas left over from meals, pea liquid and/or cans. However, in four other enterotoxin outbreaks occurring at the same time from smaller cans of peas from other canneries, a different phage type, but also in Group III, was responsible for the illness.

The fact that the same phage type had appeared in many staphylococcal intoxications associated with peas stimulated a thorough investigation at the cannery with the involvement of the Canneries Research Laboratory at Chipping Campden, England, the research department of the can manufacturing firm, the Medical Officer of Health and the Public Health Laboratory Service (*6*). A large number (3,071) of A10 cans with normal appearance was examined and 71 were found to contain mesophilic bacteria; 4 of the 71 contained *S. aureus* phage type 42E. Additionally, 14 of 91 swollen A10 cans of peas contained phage type 42E. A thorough investigation at the plant showed that the peas received adequate heat treatment, and the cooling water during the time the peas were canned was highly chlorinated (1-2 ppm, occasionally up to 4-8 ppm of free chlorine) (*7*). Swabbing the exterior of A10 cans during production revealed no *S. aureus*. However, when A10 cans were no longer produced in the canning season, No. 1 tall cans (about 0.27 kg) were swabbed. Some of the swabs from No. 1 cans yielded *S. aureus* phage type 42E. Examination of post-processing can handlers revealed that 9 of 17 were *S. aureus* carriers and one of them was a carrier of *S. aureus* phage type 42E. Further investigation revealed that this carrier had recurrent boils on her back and forearms for a long time, but the boils were not apparent and the employee had not, therefore, been removed from the production line. The *S. aureus* carrier had been employed while the implicated cans were being processed, and one of her jobs was unloading the wet and slightly warm cans from the retort crates (*24,79,80*).

Many of the A10 cans showed leakage when tested by vacuum or by pressure of the sterile cans, 10% leaked, and of the contaminated cans, 50%. Leakage was most prevalent at the side seam-double seam junction. At the time of the outbreaks, there was an unduly high percentage of "blown" cans of peas - 2.3%, Bashford et al. (*24*); 2.5%, Anonymous (*7*) - which may be indicative of inadequate seaming. Many of the contaminated cans, however, were not found to have leaking seams by conventional pressure or vacuum tests. Microleakage during cooling was, therefore, suspected. During at least two outbreaks, it was observed that the implicated cans of processed peas had no obvious signs of spoilage such as gas production, and the peas had a normal appearance and taste when eaten (*5*). As a result of one of the outbreaks a legal action was filed against the company (*5*).

This investigation showed conclusively that handlers of heat-processed containers can contaminate the contents. The organisms come from hands onto the wet surface of the containers and are then drawn into the interior through the leaks as the containers cool.

SALMONELLOSIS ARISING FROM PPL [1934-1964]

Nine outbreaks of salmonellosis arising from PPL are shown in Table 6. In each incident, a *Salmonella* sp. was isolated from food or patients (in five incidents from both). Most of the food involved was meat (corned beef, pork, ham and mutton); sardines and spinach were the other implicated foods. With one exception, all the canned products were imported, mainly to the United Kingdom. A total of five deaths were recorded from three of the earlier outbreaks (two from sardines in 1934, two from mutton in 1936 and one from luncheon meat in 1954). In only two of these outbreaks, information was recorded that the original can had been examined. In the first, *Salmonella typhimurium* was recovered from a can of Yugoslavian luncheon meat associated with illness in Germany (*58*) and from other cans of the same lot. The cans had been made by the food processing company, and evidence of poor seaming was apparent at both ends of the 102 cans examined. In the second outbreak, *Salmonella onderstepoort* was isolated from the remains of corned beef left in a can and from a carving knife after the food had been implicated in illness in England or Wales in 1962. In the 1936 mutton outbreak, the storekeeper of a Welsh institution opened 27 apparently sound cans for 763 inmates. One other can of the same lot was swollen and its decomposed contents had been destroyed in the furnace before analysis could be made. Thirty-one inmates suffered from salmonellosis and most of these were from two wards; this would indicate that probably one of the 27 cans which appeared sound was contaminated.

TYPHOID FEVER ARISING FROM PPL [1948-1964]

Six outbreaks of typhoid fever that can be attributed to PPL of canned corned beef or ox tongue are listed in Table 7. All the meat was imported to England or Scotland from Argentina. However, it was only after the last recorded outbreak (Aberdeen, 1964), with its great impact on the public and epidemiologist alike, that typhoid resulting from PPL of canned meat was established beyond doubt. Subsequent retrospective evaluation of epidemiological and laboratory data helped determine that the other listed outbreaks were caused by typhoid-infected cans probably contaminated through unchlorinated cooling water. Since the Aberdeen typhoid

TABLE 6. *Salmonellosis arising from PPL.*

Canned food	Year	Etiological agent	No. of cans infected	Weight of contents in can (g)	No. of cases	Amount of original product eaten by each victim (g)	Country where food eaten	Country of origin of cans	References
Sardines in tomato sauce	1934	*S. typhimurium*	1-5	4-6 sardines 6" long. Probably 453.6 (1 lb)	18	approx. 40-320	Trinidad	United States	*(72)*
Mutton	1936	*S. enteritidis*	probably 1	2721.6 (6 lb)	31	88	Wales	Argentina	*(53)*
Ham	1950	*S. wien*	?	?	?	?	England or Wales	France	*(33)*
Luncheon meat	1954	*S. typhimurium*	1	300	4	75	Germany	Yugoslavia	*(58)*
Luncheon meat	1958	*S. typhimurium*	?	?	4	?	England or Wales	The Netherlands	*(37)*
Corned beef	1955	*S. newport*	1	2721.6 (6 lb)	25	109	England or Wales	South America	*(34)*
Corned beef	1962	*S. onderstepoort*	1	2608.2 (5 3/4 lb)	44	59	England or Wales	South Africa	*(89)*
Spinach	early 1960s	*S. paratyphi* B	?	?	2	?	England	England	*(28)*
Chopped pork	1964	*S. reading*	probably 1	1814.4 (4 lb)	55[1]	101	Scotland	Hungary	*(31)*
Total	1934-1964	9 outbreaks			> 183		5 countries (Europe, Caribbean)	9 countries (Europe, South America, North America, Africa)	

[1]Number eating from original infected can = 18 or more

TABLE 7. *Typhoid fever arising from PPL.*

Canned food	Year	Etiological agent	No. of cans infected	Weight of can (g)	No. of cases	Amount of original can eaten by each victim (g)	Place where food eaten	Place of origin of cans	References
Corned beef (probable)	1948	*S. typhi* Vi-phage type 34 and type A	?	?	135	?	Oswestry, England	probably Argentina	(*1,27,73*)
Ox tongue	1954	*S. typhi* Vi-phage type El	1	2721.6 (6 lb.)	33[1]	118	Pickering, England	Establishment 1A, Rosario, Argentina	(*52,84*)
Corned beef	1963	*S. typhi* Vi-phage type A	1	2721.6 (6 lb.)	24[2]	113	Harlow, England	Establishment 25, Argentina	(*22,73,87*)
Corned beef	1963	*S. typhi* degraded Vi strain	deduced 1	2721.6 (6 lb.)	27[3]	124 (deduced)	South Shields, England	Establishment 25, Argentina	(*73,87*)
Corned beef	1963	*S. typhi* Vi-phage type El	deduced 1	2721.6 (6 lb.)	23[4]	151 (deduced)	Bedford, England	Establishment 25, Argentina	(*73,87*)
Corned beef	1964	*S. typhi* Vi-phage type 34 (some isolates mutated to type A)	probably 1	2721.6 (6 lb.)	515[5]	136	Aberdeen, Scotland	Establishment 1A, Rosario, Argentina	(*1,61,64,65*)
Total	1948-1964	6 outbreaks			759		England and Scotland	Argentina	

[1]Number eating from original infected can = about 23
[2]Number eating from original infected can = probably 24
[3]Number eating from original infected can = about 22
[4]Number eating from original infected can = about 18
[5]Number eating from original infected can = 12 to greater than 25, median about 20

outbreak was the most thoroughly investigated, we have listed the salient conclusions drawn from the investigation. These are: (*a*) the most probable cause of typhoid fever in Aberdeen in 1964 was canned corned beef containing *Salmonella typhi*, (*b*) this organism gained entry into the can from unchlorinated river water used for cooling after the heat processing, (*c*) the infected canned corned beef was a product of Argentina establishment 1A at Rosario sold in an Aberdeen supermarket between the 7th and 9th of May, 1964, (*d*) cans may contain typhoid bacilli without swelling, (*e*) typhoid bacilli and coliforms may grow together without swelling the can, (*f*) there was no evidence that the can was contaminated by a carrier after opening, (*g*) the infected corned beef contaminated a slicing machine and the slicing machine contaminated other meats in which *S. typhi* multiplied because of the lack of refrigeration and (*h*) the infection from corned beef and cross-contaminated cold meat products lasted till May 23, 1964 when the supermarket was closed; the majority of victims were infected from that source. A total of 507 residents and eight visitors of 35,000 at risk eventually contracted typhoid fever (*65*).

Investigations at the manufacturing plant revealed that chlorination apparatus had been out of use for some 14 months. The cans were cooled in unchlorinated river water obtained downstream from Rosario, a city of 600,000, which discharged raw sewage into the river (*93*). In most cases, *S. typhi* Vi-phage type 34 and some Vi-phage type A were isolated (*1*).

It was postulated that temporary microleaks had occurred permitting *S. typhi* to enter the cans, and it subsequently multiplied anaerobically without producing gas and without causing any organoleptic change in the meat. Because of the small size of a microleak, which may be 1 μl or less, there is no appreciable loss of vacuum in the can, and the possibility of a chance inoculum containing a single cell, and thus yielding a pure culture is not remote (*61*). Also, *S. typhi*, when mixed with *Escherichia coli* and *Enterobacter cloacae* and inoculated into canned corned beef, will exhibit strong selective advantages over the latter organisms (*1*).

In 1963, the year before the Aberdeen outbreak, three other incidents occurred in England (Harlow in May, South Shields in June, and Bedford in October), also from corned beef. Although different phage types were identified with the *S. typhi* isolated, all cans were traced to the same manufacturing establishment in Argentina where unchlorinated river water was used to cool the cans. Significantly, an outbreak of typhoid fever had occurred upstream of the factory in May and June of 1962, the very time when the cans were being manufactured. As a result of these three episodes, British health officials requested at the end of 1963 that distributors recall all 6-lb. cans of corned beef produced on or after May 30, 1962. The recalled cans examined showed that spoilage was four time greater than normal, but no salmonellae were found (*73*). Unfortunately, the recall did not prevent the 1964 outbreak in Aberdeen.

In a still-earlier episode involving canned ox tongue in Pickering in 1954, 33 people were ill and four died. In this episode, the possibility of PPL-mediated food poisoning from contaminated cooling water through microleaks was postulated for the first time (*52*). The manufacturing plant (Establishment 1A, Rosario, Argentina) had used untreated river water for cooling. After the Pickering episode, the cooling water was chlorinated, but the chlorinating system broke down in 1963, and again a can was found contaminated with *S. typhi* (*60*); the manufacturing plant, establishment 1A, was the same one that produced canned corned beef sold in Aberdeen in 1964 (*1*).

Even at this early stage in the epidemiology of food poisoning from PPL it was recognized that detection of *S. typhi* in other cans from an implicated lot would be rare. Couper et al. (*52*) knew that Sandiford (*74*) had isolated *S. typhi* from a can of sterilized cream after complaints had been made about the product; 955 cans of the same lot and 30 other lots were subsequently examined, and, although 17% were contaminated with other organisms, *S. typhi* was not recovered again. A definite can defect had been found — a knocked down flange — which would allow penetration of microorganisms from the contaminated well water used for cooling, but no case of illness had been reported.

The last outbreak to be discussed under the section on *S. typhi* PPL is the earliest one recorded. It occurred at a hospital in Oswestry in 1948, and seven persons died. The original investigators did not link a canned product to the outbreak [milk was the suspected source of the disease; Bradley et al. (*27*)]; but retrospective work by Anderson and Hobbs (*1*) showed that corned beef was on the menu on August 30, 1948, the day that the infection most likely began, and that the can probably came from Argentina. The most significant evidence to link this outbreak with an Argentinian cannery, however, was that the phage types isolated from patients, types 34 and A, were identical to those found in patients suffering from typhoid in the Aberdeen incident. Type 34 is not indigenous to the United Kingdom, but is found in Spain and Latin America (*1*).

ILLNESS FROM *SALMONELLA* AND AMOUNT OF CONTAMINATED FOOD CONSUMED

One further point to be gleaned from typhoid and salmonellosis resulting from canned meat products is the number of persons ill per can and, therefore, the approximate amount of food eaten by the ill people. It is known that only single cans were involved in two of the typhoid outbreaks (ox tongue, 1954; corned beef, 1963) and three of the salmonellosis outbreaks (luncheon meat, 1954; corned beef, 1955 and corned beef, 1962). It was, furthermore, postulated on epidemiological evidence that one can was involved in the 1936 canned mutton outbreak (see section on salmonellosis arising from PPL) and on clinical evidence that single cans caused the 1964 chopped pork salmonellosis and 1964 corned beef typhoid outbreaks. In these last two, information on incubation periods and seriousness of illness was obtained. It was assumed that the original can would be more infected than other meat cross-contaminated through human handling or slicing machines; thus, those infected from the meat of the can which had PPL would be expected to have the shortest incubation periods and the most severe signs and symptoms. In the case of salmonellosis from chopped pork, Burnett and Davies (*31*) showed that 18 or more persons fitted this category (incubation period: 8-36 h) and would probably be those who ate the luncheon meat from the contaminated 4-lb. can. In the Aberdeen typhoid outbreak Walker (*93*) stated that individuals who ate the original sliced meat had a very short incubation period for typhoid, the meat being consumed between May 7 and 9 and the first cases becoming ill on May 12 to 15. Between 12 and 25 of these people had histories of eating corned beef or unspecified cold meat from the incriminated supermarket (*61*), and, although there may have been others, at least these probably ate from the originally infected meat; only one can of corned beef was assumed to be involved. As can be seen from Tables 6 and 7, the amount of meat eaten by infected individuals ranged from 59 to 136 g (deduced amounts excepted, see below) with a median of 105 g. Because of the similarity of the South Shields and Bedford outbreaks to the Harlow episode, a single can was deduced to have been involved in each of these, and the deduced amount of corned beef per victim was 124 g and 151 g, respectively, for each outbreak.

In the earliest outbreak of PPL salmonellosis recorded (from sardines in tomato sauce in 1934), the contents of five cans were served for lunch and dinner to 21 persons, 18 of whom became ill and two died. The three persons not ill, and the nine mildly ill ate between ½ and 1 sardine for lunch only. The seriously ill patients ate between one and four sardines (mean, two); all but one of these ate sardines both at lunch and again 7 h later at dinner. One of the persons who died ate four fish, two at lunch and two at dinner. *Salmonella typhimurium* was isolated from the pooled contents of the five cans and from the patients. It would seem that at least one of the five cans was contaminated with the *Salmonella* and when the contents were mixed, the organism would come in contact with most of the fish. Thus, most people eating were infected. However, those eating larger amounts at lunch, or eating sardines at both lunch and dinner became seriously ill, indicating that the more fish eaten the more organisms were consumed, and that *Salmonella* probably increased in number during the afternoon storage at room temperature. Each sardine weighed about 80 g. Therefore, approximately 40 to 320 g were consumed by ill persons. Unknown amounts of sauce were also consumed.

TYPE E BOTULISM ARISING FROM PPL [1934-1978]

The low heat resistance of spores of *C. botulinum* type E ensures that they will be destroyed by moderate heat

processing of high moisture foods. Thus, type E botulism from canned foods strongly indicates that PPL was the cause of such food poisoning. The frequency of *C. botulinum* type E in raw fish accounts for the source of contamination in the canning plant, and since this type has no proteolytic activity, the toxic product is unlikely to be rejected by the consumer. Other *C. botulinum* toxin types may enter cans by PPL, although illness has not been reported. Type C toxin has been found in a leaking can of tuna not associated with an outbreak (*7,63*). The frequency of types A and B in agricultural products suggests that they too may at times enter cans by PPL, but their strong proteolytic and aerogenic activity would probably spoil the food, thus preventing consumption.

Three outbreaks due to type E have been recorded (Table 8). In 1934, in New York State, one of three patients died after consuming canned sprats imported from Germany; the victims had eaten the product although the can was bulged and gas escaped on opening. Hazen (*57*) recognized that the outbreak was caused by *C. botulinum* that was not type A, B or C. It was subsequently named type E (*56*).

In 1963, in Michigan, two of three patients died following consumption of tuna which tasted "all right." It had been canned 2 months previously in the United States. The can causing the outbreak was one of 2,592 packed on January 8, 1963; a second can from this lot found in a retail store was swollen and contained type E toxin. Six codes produced in the same factory in 3 months, encompassing 650,000 cans, were examined by the Food and Drug Administration of the U.S.A., and 3,300 (0.5%) were found abnormal. The abnormalities consisted mainly of defective closures of lids put on at the cannery. In all, 22 cans were found to contain *C. botulinum* type E, and many other cans were contaminated with non-toxinogenic organisms. *C. botulinum* type E was isolated from four sites on can handling equipment which was in contact with the canned tuna after heat processing, and from raw fish in the plant.

In July, 1978, two of four people died in England following consumption of salmon from a single can. The can was one of a lot of about 14,000 cans produced in a cannery in Alaska on July 14, 1977. *C. botulinum* type E apparently gained entrance after processing through a small hole in the rim at one end of the can. The cause of the perforation in the can is unknown, as discussed previously, but the source of contamination may have been gloves or aprons used by workers butchering the raw product. The clothing was dried on cans that were cooling. The incident appears to be unique; other cans in the same lot were not found to be defective.

CLOSTRIDIUM PERFRINGENS ENTERITIS ARISING FROM PPL [1949-1958]

C. perfringens was the etiological agent in three food-poisoning incidents associated with canned meat products (Table 9) in England and Wales between 1949 and 1958. Although the cans were opened just before consumption, the PPL nature of contamination may be debatable, because the heat resistance of the spores, and the heat treatment history of the foods are not known. Thus there was a possibility that the presence of *C. perfringens* in the cans could have been due to either under-processing or post-processing leakage. However, in at least one of the three incidents PPL seems likely. In England in 1949, a can of pork eaten within 20 min of opening caused food poisoning, and large numbers of *C. perfringens* were found in the remainder of the meat. Interestingly, two other cans from the identical lot of canned pork caused staphylococcal food poisoning in widely separated areas of England, and both of these were due to the same phage type of *S. aureus*, which was

TABLE 8. *Type E botulism arising from PPL.*

Canned food	Year	No. dead/No. ill	Country where food eaten	Country of origin of cans	Type of can defect	References
Sprats	1934	1/3	United States	Germany	?	(*57*)
Tuna	1963	2/3	United States	United States	defective closure of canner's end	(*23,55,62,70*)
Salmon	1978	2/4	England	United States	cut on rim	(*12,13,14,15,16,17, 18,19,45,68*)
Total	1934-1978	5/10	United States and England	United States and Germany		

TABLE 9. Clostridium perfringens *enteritis arising from PPL.*

Canned food	Year	No. of cases	Country where food eaten	Country of origin of cans	References
Pork	1949	?	England	Europe	(*32,71,82*)
Stewed steak	1956	6	England or Wales	Ireland	(*35*)
Ox tongue	1958	89	England or Wales	Czechoslovakia	(*37*)
Total	1949-1958	> 95	England or Wales	3 European countries	

found in patients and remnants of food. Since at least two cans of this lot caused PPL food poisoning due to *S. aureus* it is likely that this *C. perfringens* incident was also due to PPL.

PPL FOOD POISONING ARISING FROM MISCELLANEOUS AND UNDETERMINED AGENTS [1921-1979]

A number of apparent PPL food poisonings occurred where organisms were not looked for, or found, in cans or clinical specimens, or where organisms isolated are not universally considered to be capable of causing illness. These are documented in Table 10.

Streptococci, micrococci and/or coliforms

In the United States, Buchbinder et al. (*30*) described a food poisoning outbreak caused by evaporated milk affecting 74 individuals in a children's institution. *Streptococcus faecalis* and micrococci were isolated from the milk; cans were rusted, and leakers, springers and

TABLE 10. *Miscellaneous and undetermined agents associated with PPL food poisoning.*

Etiological agent	Canned food	Year	No. of cases	Incubation period (hours)	Country where food eaten	Country of origin of cans	Type of can defect	References
Streptococcus faecalis and micrococci	evaporated milk	mid 1940s	74	2-7	United States	probably United States	corrosion	(*30*)
Coliforms	corned beef	1954	2	3-6	England or Wales	?	?	(*34*)
Coliforms and fecal streptococci	corned beef	1956	3	3	England or Wales	Tanganyika	?	(*35*)
Aerobic colony count, 85,000/g Anaerobic colony count, 125,000/g *Streptococcus faecalis* and *Clostridium butyricum* dominant organisms	whole kernel corn	1973	4	3/4	Canada	?	leak at seam juncture	(*10*)
Aerobic colony count, 20,000,000/g	jugged hare	1974	1	?	Canada	?	pin hole leak with with corrosion	(*20*)
Suspect mold	tomato juice	1973	2	24	Canada	?	hole in lid	(*10*)
Suspect mold	pea soup	1974	1	2	Canada	?	case-cutter damage	(*20*)
Suspect mold	creamed mushrooms	1974	1	2	Canada	?	leak in can	(*20*)
Suspect mold	cream of chicken soup	1976	1	1	Canada	Canada	case-cutter damage	(*77*)
Suspect mold	apple juice	1976	1	?	Canada	?	can with defects	(*77*)
Suspect mold	baked beans	1976	1	5	Canada	?	case-cutter damage	(*77*)
?	salmon	1921	5	3	England	?	?	(*75*)
?	salmon	1976	2	2	Canada	?	case-cutter damage	(*77*)
?	salmon	1978	2	13	England	U.S.S.R.	?	(*46*)
?	salmon	1979	2	12	England	U.S.S.R.	?	(*49,50*)
?	sardines	1922	1	a few hours	Scotland	?	?	(*75*)
?	corned beef	1950	?	?	United Kingdom	?	?	(*83*)
? (dead cocci in food)	corned beef	1956	2	2	England or Wales	Tanganyika	?	(*36*)
?	corned beef	1956	2	4	England or Wales	The Sudan	?	(*36*)
?	corned beef	1956	3	5-13	England or Wales	?	?	(*36*)
?	corned beef	1956	3	2-3	England or Wales	?	?	(*36*)
?	luncheon meat	1950	?	?	England or Wales	?	?	(*33*)
?	luncheon meat	1974	1	5 min.	Canada	?	can with leaks	(*20*)
?	peaches	1974	3	2	Canada	?	cans of same lot with leaks	(*20*)
?	vegetable soup	1974	1	16	Canada	?	case-cutter damage	(*20*)
?	pork and beans	1975	1	2	Canada	?	cans of same lot with leaks	(*77*)
?	tomato soup	1975	1	4½	Canada	?	case-cutter damage	(*77*)
?	tomato juice	1976	1	17	Canada	Canada	case-cutter damage	(*77*)
?	tomatoes	1976	1	4½	Canada	?	holes in embossed code	(*77*)
?	peas	1976	1	3	Canada	?	case-cutter damage	(*77*)
?	cream style corn	1976	1	1	Canada	?	case-cutter damage	(*77*)
?	macaroni and cheese	1975	1	5-15 min.	Canada	?	punctured lid	(*77*)
?	milk with baby formula	1975	1	3	Canada	?	leak in side seam	(*77*)
Total	33 incidents	1921-1979	> 126		5 countries (North America, Europe)	4 countries (North America, Africa, Europe) many others not known		

swells were found in the same batch. A few years later in England in 1954 and 1956, coliforms and coliforms and streptococci, respectively, were isolated from cans of corned beef involved in two outbreaks.

High bacterial counts

Whole kernel corn was the vehicle in an incident in Canada in 1973; four persons were ill. The contents of the can had an aerobic colony count of 85,000/g and an anaerobic colony count of 125,000/g. *C. butyricum* and *S. faecalis* were the dominant organisms. The implicated can had a leak at the side seam-double seam juncture at the canner's end. Jugged hare caused one person to be ill in Canada in 1974. The food had an aerobic colony count of 20,000,000/g; other cans of the same code and of another code showed pin hole leaks and corrosion.

Suspect mold

In six food-poisoning incidents in Canada between 1973 and 1976, mold was suspected of being the etiological agent. Mold was isolated from a can of tomato juice after two persons had consumed it and were ill; a minute hole was detected in the implicated can's lid. Five single cases were associated with canned fruit and vegetable products and cream of chicken soup. These had case-cutter damage or other leaks through which the mold gained entry into the products.

Undetermined agents

Very little is known of the etiology of two early outbreaks. A freshly opened can of salmon in England affected five persons in 1921. In a thoroughly investigated case in 1922 in Scotland, one child ate sardines in oil which caused illness a few hours later, then death. In neither incident did microbiological or chemical analysis yield any positive results. Over 50 years later canned salmon in Canada made two persons ill after the contents had been contaminated through case-cutter damage; the agent was not determined.

In 1950 and in 1956 in England and Wales, five incidents were related to freshly opened cans of corned beef. Presence of living organisms in food or patients, however, was not reported, though in one outbreak dead cocci were seen microscopically on a slide of a meat smear. Luncheon meat caused two outbreaks; in one of these in 1974 the meat smelled badly, and the implicated can leaked, but bacteriological analysis was not reported.

Eight single cases of illness and one outbreak of three persons from canned fruits, vegetables and macaroni and cheese occurred because of leaks or punctures in the cans. Canned milk with infant formula soured because of a leak in a side seam and one child became ill; no analysis was done.

CONCLUSIONS

PPL in cans is not as well recognized today as it should be, although recent reports are beginning to indicate the extent of the problem. In Australia, PPL accounted for 40% of lots with spoiled cans between 1968 and 1977 (*67*). In the United States the level was even higher; 60% between 1960 and 1977 (*76*). No illness arising from PPL is mentioned by these authors, but the potential for food poisoning is stressed by Murrell (*67*). That PPL is a hazard to the public is clear from the 154 incidents described in this review. Although it can be claimed that most of the documented outbreaks took place several decades ago, PPL does cause illness today; 12 outbreaks of staphylococcal intoxication occurred in the last 2 years and the 1978 *C. botulinum* incident in England caused two deaths and considerable disruption to the North American salmon industry. Most of the PPL outbreaks, especially the earlier ones, were recorded from the United Kingdom (72.7%). Since 1972 such illnesses have also been reported in Canada, resulting in 17.5% of the total listed here. The five other countries where such data have been published amount to no more than 9.8%, combined. The small percentage for the United States (1.9%), where PPL accounts for 60% of lots with spoiled cans (*76*) is not understood. With the exception of two well investigated botulism outbreaks (in 1934 and in 1963), and a *S. faecalis* incident in 1948, PPL food poisoning had not been reported there.

Since usually only one can is involved in each outbreak, the retrieval of these cans, and collection of the incriminated food and clinical specimens is very important. If the episodes listed in this review represent only a fraction of those occurring, then appropriate action must be taken to reduce PPL, not only at the manufacturing level but at the retail store as well.

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Regulatory Aspects of Post-Processing Microbiological Contamination of Low-Acid Canned Foods

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ABSTRACT

Various Canadian regulations pertinent to post-processing microbiological contamination of low-acid canned foods are reviewed. As an example of the dilemma presented to the regulatory agency, a typical case history is presented. The case concerns a canned fish product suspected of causing staphylococcal intoxication in two persons. The results of the following investigation are presented and the reader asked to be the judge.

Other papers (*3-7*) have verified the fact that microbiological recontamination of low-acid canned foods, following heat processing, occurs with alarming frequency. Such recontamination has not only resulted in considerable product spoilage but has caused numerous outbreaks of food poisoning, some of which resulted in deaths. A regulatory agency, while primarily concerned with the public health aspects, must also be concerned with spoilage by microorganisms.

CANADIAN FOOD AND DRUG ACT AND POST-PROCESSING MICROBIOLOGICAL CONTAMINATION

Growth of microorganisms in low-acid foods in hermetically sealed containers is considered to be a violation of the Food and Drug Act and most probably Section 4 of the Act. Section 4 states:

"No person shall sell an article of food that (*a*) has in or upon it any poisonous or harmful substance, (*b*) is unfit for human consumption, (*c*) consists in whole or in part of any filthy, putrid, disgusting, rotten, decomposed or diseased animal or vegetable substance, (d) is adulterated or (*e*) was manufactured, prepared, preserved, packaged or stored under unsanitary conditions."

A canned food found to contain pathogenic organisms or toxic substances resulting from microbial growth would be in violation of Section 4(a). The presence of organisms associated with feces, or for that matter any organisms, could be in violation of Section 4(e). Microbial spoilage, with or without the presence of viable organisms, is dealt with by Section 4(c).

The unsanitary conditions referred to in Section 4(e) are defined as those conditions or circumstances which might contaminate a food, drug or cosmetic with dirt or filth or render the same injurious to health. Thus, containers having defective seams or seals could permit the re-entry of microorganisms, even though there may be no evidence that re-entry had occurred, would also be in violation of Section 4(e). Unsanitary conditions are also the subject of Section 7, in that "no person shall manufacture, prepare, preserve, package or store for sale any food under unsanitary conditions."

With the exception of that for "Sterilized Milk," the regulations under the Act contain little further guidance with respect to such contamination. Sterile milk, regulation (B.08.007), must be heated to at least 100 C for a length of time sufficient to kill all organisms present and resulting in no concentration or appreciable loss of volume. Canned vegetables, (B.11.002), and mushrooms, (B.11.003), must be packed in hermetically sealed containers and heat processed. While the term "heat processed" lacks the required specificity, the absence of a hermetic seal could be considered a violation of these regulations. Regulation B.14.013 states that no person shall sell a meat, meat by-product or preparation thereof, packed in hermetically sealed containers, unless it has been heat processed after or at the time of sealing at a temperature and for a time sufficient to prevent survival of any microorganisms capable of producing toxins. Obviously this was designed for protection from *Clostridium botulinum* and has little application to post-processing contamination.

The Fish Inspection Act of Canada and its Regulations add very little to the regulatory aspects of this problem for canned fish or fish products. It is stated that swollen or bulging, improperly sealed or otherwise defective containers cannot be exported or imported. Canned fish must be sterilized by a method approved by the Minister. Sterilized is defined as heat treatment to prevent spoilage and to destroy all pathogenic organisms. Similarly, the Meat Inspection Act and Regulations deals with the heat processing and not what could happen after such processing.

INCIDENCE OF POST-PROCESSING CONTAMINATION

As has been discussed in other papers (*3-7*), post-processing contamination occurs at a significant level under conditions believed to be consistent with good manufacturing practice (GMP), that is, the present state of the art. This gives rise to two questions: what is the level and what position should a regulatory agency take with respect to this level? Can a regulatory agency

realistically expect perfection, that is, the complete absence of such contamination even though the present technological practice cannot attain that goal?

Pflug (unpublished) has stated that his studies with blown cans recovered at the retail level showed a frequency as high as 78 swells per 100,000, with the mean being about 10 per 100,000. Since his recovery of swollen cans was somewhat less than 100%, somewhere between 50% and 75%, these frequencies should be factored accordingly. A further factoring would result from the fact that not only gas-forming organisms contaminate cans after processing, but also non-gas-forming organisms. It is reasonable to assume that the incidence of post-processing contamination by non-gas-forming organisms could be at least equal to that of the gas-forming organisms. Also, slightly swollen cans (flippers and springers) are not easily detected by untrained or inexperienced persons, such as clerks in chain stores. Therefore, considering all of the above, it is reasonable to assume a post-processing contamination level of about 0.1%, that is 1 per 1,000.

Most cases of post-processing contamination are brought to the attention of a regulatory agency via consumer complaint. The evidence resulting from the subsequent investigation is frequently fragmentary and incomplete. The difficulty in such investigations is compounded by the fact that the incidence of contaminated or defective containers is usually quite low, 5% or less, hence a large number of samples must be analyzed to obtain sufficient evidence. This is further complicated by the fact that the product usually has been processed some time before the complaint, that is, it has been in general distribution for some time, hence the container population open to sampling has been reduced. Definitive proof is the exception rather than the rule. To illustrate this we will present a typical case, giving you the facts as they were received. We would like you to be the judge and answer the following questions: (*a*) Should the product be recalled, and if so to what level? (*b*) Would you advise the issuing of a public health alert? (*c*) What other compliance or legal action should be taken?

Remember, time is an essential element whenever public health is concerned; frequently decisions must be made well before any investigation can be carried out exhaustively.

CASE HISTORY - FOOD POISONING RESULTING FROM POST-PROCESSING CONTAMINATION OF CANNED FISH

Complaint

A provincial health authority reported that two persons had succumbed to staphylococcal intoxication 2 ½ h after consuming part of the contents of a 7-oz can of a fish product. The diagnosis was made by a physician. This was the only food item eaten in common by the two persons and not eaten by other persons present at the meal and who did not become ill. Analysis of the remaining product showed a confirmed *Staphylococcus aureus* count of 5.7×10^6, the presence of heat stable DNase and 20 ng of enterotoxin A per gram. The canner's double seam on the complaint can was very loose, apparently having received little or no pressure during the second roll operation. Evidence of this was a seam thickness well above the maximum permitted in the can supplier's specifications, the absence of any pressure ridge and a tightness rating of zero.

Analysis of product having the same code

On the same day as the complaint was received, 5 cans of the product bearing the same code as the complaint can were obtained from the same retail outlet and analyzed at a regional laboratory.

The results of the container integrity evaluation and double seam measurements of the 5 cans are given in Table 1. All five leaked at pressures of 5 inches of mercury or below, as designated in the column headed, "vacuum integrity test." The tightness rating of the canner's seams were all zero, and there was no evidence of a pressure ridge. For comparison, the same measurements and evaluations of the can manufacturer's

TABLE 1. *Integrity evaluation and double seam measurements of the 5 cans obtained following the complaint.*[a,b]

		Canner's double seam					Manufacturer's double seam				
Can	Vacuum integrity test	Body hook	Cover hook	Overlap	Pressure ridge	Tightness rating	Body hook	Cover hook	Overlap	Pressure ridge	Tightness rating (%)
A	Leaked	(59)	(58)	32	Absent	0	83	74	44	Good	100
	(3" of Hg)	76	(64)	36			87	82	53		
B	Leaked	(67)	(56)	30	Absent	0	78	81	41	Good	100
	(4" of Hg)	72	(59)	39			80	83	56		
C	Leaked	(63)	(57)	30	Absent	0	76	80	50	Good	100
	(1" of Hg)	(67)	(62)	39			87	83	53		
D	Leaked	(62)	(59)	30	Absent	0	78	75	49	Good	100
	(4" of Hg)	(66)	(65)	35			84	84	55		
E	Leaked	(65)	(58)	37	Absent	0	82	82	53	Good	100
	(5" of Hg)	72	(62)	43			86	85	57		
Seam thickness				69 to 79					46 to 51		
Seam height				104 to 115					133 to 128		

[a]All measurements are × 0.001 Inches.
[b]Figures enclosed in brackets are outside canner's double seam specifications.

double seam are given. It is evident that all five canner's seams were quite loose, similar to that found in the complaint can.

The 5 cans were opened aseptically and the contents analyzed microbiologically and for pH. The results are presented in Table 2. The sterility testing was carried out by the aseptic transfer of approximately 2 g of a combination of brine and scrapings from the product surface to quadruplicate tubes of each of sterile bromocresol purple glucose broth (BCP broth) and sterile modified cooked meat broth (MCM broth). Duplicate inoculated tubes of each broth were incubated at 35 and 55 C. All tubes of the MCM broth for can D at both temperatures were positive, in that they showed evidence of microbial growth, whereas only those of the BCP broth at 35 C were positive. None of the tubes from the other cans were positive. When the positive tubes were streaked on liver veal agar (LVA) plates and incubated aerobically and anaerobically at 35 C, numerous small colonies developed on the plates from the tubes at 35 C, whereas those from the 55-C tubes were negative. Wet amounts showed the organism to be a non-motile rod and further tests indicated that they were gram positive and catalase negative. No spores were observed.

In addition, 1:10 dilutions of the contents of each can were streaked directly on Plate Count Agar (PCA) plates which were incubated at 30 and 55 C and on prepared Baird-Parker (BP) plates incubated at 35 C. As can be seen in Table 2, the three colonies on the BP plate from can B was the highest observed, the remainder being one or no colonies. The plates from can D, the one having positive tubes in the sterility test, were all negative. Unfortunately the direct plating was not carried out under acceptable aseptic conditions, such as a laminar flow hood, and hence such low colony counts must be suspect. None of the contents of the cans were positive for heat stable DNase nor were the pH values different from that which was expected for the type of product.

The direct microscopic counts (DMC) were exceedingly high, varying from about 10 rods/field to 50 rods/fields with, in most instances, a few cocci. The absence of detectable viable organisms in product having a high DMC leads one to suspect either auto-sterilization or incipient spoilage. It is interesting that there was no change in the pH in product having such high DMC values.

Investigators were experiencing difficulty in locating additional samples of the product bearing the same code. Finally two cans were located and analyzed. Since no growth was observed in tubes of BCP or MCM broths inoculated with the product, the contents were judged to be commercially sterile. The contents showed DMC's of less than 1 cell/5 fields, considerably different from the previous 5 cans analyzed. The results of the double seam examinations are given in Table 3. Both the canner's double seams showed the presence of an adequate pressure ridge and had tightness ratings of 90% and 100%; however, all thickness measurements were above the maximum permitted in the canner's specifications, as were most of the height measurements. The seams were judged to be sound.

Inspection of the cannery and cannery records

The cannery is relatively small, having two single-head vacuum seamers and five small horizontal retorts. All cans are embossed with a code identifying the

TABLE 3. *Double seam measurements of 2 cans with the same code obtained from retail outlet.*[a,b]

Double seam measurement	Manufacturer's double seam		Canner's double seam	
	Can #1	Can #2	Can #1	Can #2
Thickness	0.049	0.048	(0.055)	(0.053)
	0.049	0.049	(0.052)	(0.053)
	0.049	0.048	(0.056)	0.050
Height	0.128	0.125	(0.111)	(0.112)
	0.127	0.123	0.115	(0.113)
	0.128	0.125	0.117	(0.114)
Body hook length	0.083	0.081	0.079	0.076
	0.083	0.081	0.077	0.079
	0.082	0.086	0.075	0.078
Cover hook length	0.078	0.080	(0.068)	0.077
	0.079	0.083	0.073	0.077
	0.082	0.080	(0.067)	0.076
Tightness rating	100%	100%	90%	90%
Pressure ridge	Good	Good	Good	Good

[a]All measurements are in inches.
[b]Measurements enclosed by brackets indicate values outside the canner's double seam specifications.

TABLE 2. *Microbial examination of the contents of the 5 cans obtained following complaint.*[a,b,c,d]

Can	pH	Direct microscopic examination	Sterility testing: BCP Broth 35 C	BCP Broth 55 C	MCM Broth 35 C	MCM Broth 55 C	PCA plate 30 C	PCA plate 55 C	BP plate 35 c	Heat stable DNase
A	7.2	Rods 50/field, few cocci	− ve	− ve	− ve	− ve	1	0	0	− ve
B	7.0	Rods 5 - 10/field	− ve	− ve	− ve	− ve	0	0	3	− ve
C	7.0	Rods 50/field, cocci 5 - 10/field	− ve	− ve	− ve	− ve	0	0	1	− ve
D	7.0	Rods 50/field, few cocci	+ ve	− ve	+ ve	+ ve	0	0	0	− ve
E	6.9	Rods 50/field, few cocci	− ve	− ve	− ve	− ve	1	0	1	− ve

[a]Numbers represent number of colonies observed on the plates.
[b]All positive tubes streaked on LVA plates, incubated aerobically and anaerobically, 35 C.
[c]Plates from positive BCP and MCM tubes at 35 C showed numerous small colonies, those at 55 C were negative.
[d]Wet mounts showed non-motile rods. Colonies were catalase-negative. Organisms gram positive rods having lengths from 1.5 to 2.0 micrometers. No spores observed.

establishment, the day and year processed and which seamer was used for the seaming.

Preparation of the fish and filling of the containers was mostly manual. The cleaned, skinned and trimmed fish was cooked for 20 to 25 min in live steam, then shredded, with bones, skin and any foreign matter being removed. The shredded fish was manually placed, by weight, into the cans, a sodium hexametaphospate/sodium chloride tablet pressed into the interior of the product and a parchment liner placed on top and then the can was sealed. The cans, received in paper bales, were placed directly on the filling conveyor without inversion or cleaning in any manner.

After seaming, the cans fell into the retort baskets. To prevent injury to the filled, sealed cans, the baskets were immersed in a tank of warm water. The cans were not washed before entry into the baskets. Since the water in the tanks containing the baskets was changed infrequently, it would quickly become contaminated with product adhering to the exterior of the can. Since the water used was obtained directly from the municipal water supply and received no further treatment before or during its use in the buffer tanks, one could expect considerable microbial growth to be present in the tanks. The vacuum inside the cans would certainly assist in the entry of this contaminated water into the contents, especially in the case of defective seams.

The retorts were loaded and unloaded manually. The scheduled process calls for an initial internal temperature of 70 C, a sterilization temperature of 240 F and a sterilization time of 75 min for 14-oz cans and 65 min for 7-oz cans. The initial cooling was carried out in the retort, using municipal water which had received no further treatment, e.g., chlorination. Further cooling and drying of the container exterior was carried out on conveyors following the retort cooling and the product was packed "bright" into cartons. The product was transported to another establishment for labelling.

Only the one fish product was produced during the 24 days production of the product. On the 5th day, 10 retort loads were processed, 8 of 7-oz cans and 2 of 14-oz cans. According to the company, each retort basket contained 600 of the 14-oz cans or 1200 of the 7-oz cans and each retort load contains two baskets. Taking into account the number of retorts processed, the production for the 5th day was 400 cases of the 7-oz product and 50 cases of the 14 oz. However, production records show a production for that day of 734 cases of the 7-oz product and 75 cases of the 14-oz product. The difference between the two sets of figures would represent about 7 ½ retort loads, for which there was no record. The company insisted that the higher figure was correct. No explanation was given for the discrepancy.

The daily process records showed no entry for the initial internal temperatures, thus it must be concluded that none were measured. The scheduled process calls for a venting time of between 8 and 11 min but no temperature to be attained at the end of the venting time was declared. The process records show venting time was almost always 11 min, but there was no record of the temperature at the end of venting.

There was no record that heat distribution studies had been conducted nor had any heat penetration studies been done on the product for development of the scheduled process. The cook time and temperatures for the product in each can size were obtained from a bulletin issued by the Department of Fisheries, Technical Report Series N. Mar/T-76-1, Process Tables for Canned Fishery Products by A. B. Dewar and R. L. Selfridge.

The daily process record for the 5th day showed that of the 10 retort loads processed that day, two had initial and final mercury thermometer readings of 236 and 238 F, respectively, two with 239 and 240 F, respectively, and one with 238 and 240 F, respectively. The charts from the temperature recorder controllers for each of the retorts used showed all temperatures to have been at least 240 F throughout the process time for each cook. Obviously the temperature recorder controllers were not standardized to the mercury thermometers. It appears that some of the loads were processed at temperatures slightly below that designated in the scheduled process.

Both the process time and the temperature were regulated by the temperature recorder controller. The time when the steam was turned on, vent closed and commencement of the cook were taken from a wrist watch and the time for the end of the cook was calculated by adding the appropriate process time to that of when the cook began. The actual time that the steam was turned off by the temperature recorder controller was not checked nor was the accuracy of the timing device. Therefore, the process times are suspect.

The times indicated on the charts from the temperature indicator controllers in no way coincided with those in the retort log. The process times derived from the charts appeared to correspond to those in the scheduled processes as did the time intervals between loads for each retort. No reason was given by either the plant supervisor or management for the discrepancies in the temperatures or the times. The respective retort loads were not identified on the charts nor was there any indication that the charts had been reviewed by the plant supervisor or management.

GMP dictates (*1,2*) that an unprocessed low-acid product should not be maintained in a hermetically sealed container for longer than 30 min before commencement of the heat processing. The cannery management admitted to time intervals of up to 60 min. It must be remembered that during this time the product was being accumulated and held in a tank of warm contaminated water. The daily process record for the 5th day showed that the 10 loads were processed over a period of 16 h. The time intervals between loads varied from 26 min to 200 min, with only two intervals being 30 min or less, the rest varying from 76 to 200 min. Even taking into account coffee and meal breaks, it is difficult to account for the long time intervals. One seamer

operating at its rated capacity should seal enough cans to fill a retort every 30 min.

During the 24 days in which this particular product was packed, seam evaluations were carried out at 0930 and 1130 hours on only 4 of the 24 days, one of which was the 5th day. At each time 2 cans were taken, examined visually for seam defects and the canner's double seam was torn down and examined. There is reason to doubt that the seam evaluations were carried out immediately after the taking of the samples, it may well have been later in the day. The records do not indicate on which of the two seamers the cans were seamed.

The results of the double seam evaluations for 5th day are reproduced in Table 4. The canner's specifications have been included for comparison. Although two retort loads of 14-oz cans were processed that day, there is no record of any seam analysis for those containers. The figures enclosed in brackets are outside the canner's specifications, e.g., all the thickness measurements and one height. It is not known whether these figures represent single measurements for each can or an average of two or more measurements. GMP's recommend that at least three be taken, each at about 120° from the other and not to include the crossover area (*1,2*). The measurements taken in this examination, that is double seam height and thickness, and body and cover hooks lengths are not considered sufficient to adequately judge the quality of the double seam. In addition to these measurements, the crossover thickness and the overlap should be measured and an evaluation made of the juncture, the tightness and presence of an adequate pressure ridge.

TABLE 4. *Canner's seam measurements*

Double seam measurement	Canner's specification	0930		1130	
Thickness	0.047-0.051	(0.052),	(0.054)	(0.053),	(0.055)
Cover hook length	0.072-0.088	0.080,	0.081	0.081	0.083
Body hook length	0.072-0.088	0.078	0.079	0.075	0.078
Height	0.115-0.121	0.115	0.116	(0.113)	0.117

The frequency of double seam evaluations was also completely inadequate. GMP (*1,2*) dictates that a visual seam examination should be done at least every 30 min and a tear-down examination at least every 4 h of one can for each seaming head. These examinations should also be carried out at start-up of the seamer and after any prolonged stoppage or shutdown. On the 5th of July, the operations took place over 16 h with only two tear-down examinations carried out 2 h apart. No record of visual examinations was available.

Both the person in charge of quality control and the seamer operator have successfully completed an accredited course in heat processing of low-acid foods for seamer and retort operators. This course covers the essential elements of GMP for the canning of low-acid foods (*1,2*), so there is little excuse for the above deficiencies.

There was no record of verification of the accuracy of the mercury-in-glass thermometers, temperature recorder controllers or pressure gauges. During an inspection of the cannery, 9 months later, a mercury thermometer on one retort was broken and another had a separated column.

Further product analysis

No stock of the product produced during the 24 days remained in any of the company's warehouses. The company reported that this particular product sells very well, with demand being three times the supply. Efforts were continued to find additional samples of the product, not only processed on the 5th day but on any day out of the 24. Finally, 4 additional cans bearing the same code as that of the complaint were located as well as others covering process dates from the 3rd to the 8th day. All samples were obtained at the retail level. Results of the analysis of the samples are given in Table 5.

The analysis of the samples was divided between two laboratories, which gave rise to some differences as to the specific tests or analysis carried out. This is particularly evident with respect to the evaluation of the double seam and container integrity, (Table 5). The three readings for each measurement such as double seam height, thickness, etc., represent those taken at approximately 120° from each other on the same double seam. Bracketed figures are those outside the canner's specifications. With two exceptions, all containers appeared to have had good seals in that they did not leak at applied pressures below 15 psig or that they had a tightness rating of 80% or better, an adequate pressure ridge and overlap. The two exceptions were can #8, processed on the 6th day and #3 processed on the 8th day. The canner was having considerable difficulty in maintaining the double seam height and thickness specifications.

The pH values for the contents were all within the expected range of 6.7 to 6.9. No viable organisms were found during the sterility testing of the contents and all DMC's were 1 cell/5 fields or less. No further evidence of post-processing microbiological contamination was found. Neither the cans nor their contents gave results comparable to those found with the complaint can or the 5 samples taken from the same retail outlet following the complaint.

You now have all the evidence which was received for evaluation. We are sure that you would like to have more information with respect to the process, as well as additional and probably different analyses. Regrettably this is all there is. It is time for a decision — what is yours?

POSTSCRIPT

Shortly after the termination of the above investigation, another food poisoning incident, involving two persons, was reported and believed due to consumption

TABLE 5. *Evaluation of the canner's double seam of containers from product processed in July 1978 at the same cannery.*

Date processed	Can no.	Canner's double seam (× 0.01") Cover hook			Body hook			Overlap			Thickness			Length (height)			Tightness rating	Pressure ridge	Pressure integrity test
Day 3	1	80	81	74	73	(70)	(69)	51	52	48	(55)	(52)	(55)	(109)	(113)	(110)	90%	Good	N.D.
Day 4	1	76	77	76	78	77	76	51	52	47	(55)	(54)	50	(113)	(112)	(114)	85%	Good	N.D.
	2	72	77	77	78	77	81	48	51	55	(56)	(54)	(53)	(112)	(113)	(113)	80%	Good	N.D.
	3	78	75	75	72	74	74	48	47	49	(55)	(52)	(53)	(112)	(112)	(110)	100%	Good	N.D.
	4	77	75	73	81	76	77	58	48	47	(53)	(54)	51	(111)	(113)	(113)	100%	Good	N.D.
Day 5	1	77	77	76	76	79	78	51	53	50	(53)	50	50	(112)	(113)	(114)	90%	Good	N.D.
	2	(68)	(73)	(67)	79	77	75	46	45	39	(55)	(52)	(56)	(111)	115	117	90%	Good	N.D.
	3	77	80	77	80	77	77	59	56	56	(52)	(57)	—	(110)	(111)	(111)	N.D.[a]	N.D.	slow at 18 psig
	4	73	76	78	75	81	88	54	60	60	(53)	(58)	—	116	(109)	(111)	N.D.	N.D.	slow at 18 psig
Day 6	1	79	76	(61)	78	79	78	53	53	57		49 to 51		117	117	117	N.D.	N.D.	positive 20 psig
	2	74	79	(71)	80	85	80	58	59	49		″		(109)	118	117	N.D.	N.D.	positive 20 psig
	3	75	(69)	80	87	83	86	58	48	59		″		117	117	119	N.D.	N.D.	negative
	4	74	75	74	79	75	82	55	53	54		″		(112)	(110)	(109)	N.D.	N.D.	positive 18 psig
	5	77	79	76	81	84	79	57	59	53		″		(114)	116	115	N.D.	N.D.	negative
	6	76	77	76	79	(89)	84	53	61	61		″		(113)	119	120	N.D.	N.D.	negative
	7	72	76	73	80	85	83	53	59	51		″		(113)	116	118	N.D.	N.D.	positive 20 psig
	8	73	74	76	80	84	79	55	53	52		″		(111)	117	116	N.D.	N.D.	positive 9 psig
	9	73	74	75	79	83	79	53	54	50		″		(112)	118	116	N.D.	N.D.	positive 20 psig
	10	73	77	78	83	82	80	54	57	56		″		117	116	(114)	N.D.	N.D.	negative
Day 7	1	79	75	72	82	78	84	58	51	51		″		117	115	118	N.D.	N.D.	negative
	2	75	72	77	79	79	79	53	54	55		″		(114)	119	116	N.D.	N.D.	negative
	3	74	79	73	83	79	80	53	57	53		″		116	(113)	(111)	N.D.	N.D.	negative
	4	78	72	79	81	79	73	58	50	49		″		(114)	(114)	115	N.D.	N.D.	negative
	5	(68)	72	74	83	79	77	57	50	52				115	(114)	(111)	N.D.	N.D.	negative
	6	77	77	77	80	80	80	5	54	59		″		(114)	115	(111)	N.D.	N.D.	negative
	7	75	(71)	74	81	79	84	56	48	58		″		(112)	115	(114)	N.D.	N.D.	positive 14 psig
	8	(70)	76	76	78	77	81	48	54	56		″		(111	(112)	(113)	N.D.	N.D.	slow at 20 psig
	9	82	(71)	79	85	77	81	61	48	58		″		119	(112)	(113)	N.D.	N.D.	negative
	10	78	80	76	78	80	77	58	58	53		″		(113)	(112)	(112)	N.D.	N.D.	negative
Day 8	1	75	72	73	79	79	83	52	47	50	(55)	(55)	(55)	(112)	(114)	116	80%	Good	N.D.
	2	78	74	75	82	83	82	56	52	53	(56)	(55)	(56)	(114)	115	(114)	80%	Good	N.D.
	3	73	77	74	80	81	81	54	56	55		49 to 51		(112)	(111)	(111)	N.D.	N.D.	positive 1 psig
	4	76	(69)	(70)	84	75	79	57	47	51		″		116	(111)	(112)	N.D.	N.D.	negative

[a]N.D. = Not determined.

of the same product, from the same cannery and manufactured during the same month. Symptoms indicate that the illness was staphylococcal intoxication. A search of the retail stores in the same locality as the complaint produced additional samples of the product manufactured during the same period. The analysis, however, failed to reveal any post-processing contamination or defective seams.

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Effects of Germicides on Microorganisms in Can Cooling Waters

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ABSTRACT

Minimization of microbial spoilage in canned foods is accomplished by application of an appropriate thermal process and by controlling post-processing contamination by controlling the microbial population in container cooling water. The most widely used means of controlling microbial population in cooling water is with the application of germicidal solutions. The amount, kind and manner of application affect the effectiveness of germicidal solutions. This report discusses the various germicidal solutions used and their effectiveness, particularly in controlling microbial populations.

Following thermal processing, foods in hermetically sealed containers are usually cooled as rapidly as possible, using cold water. It is during this period, when hot containers are exposed to cooling water, that the initial possibility of microbial reinfection of the container occurs. To prevent microbial reinfection it is important that the container be closed well and that the thermally processed hermetically sealed container be exposed to water which has a low microbial load. This requirement for water containing low numbers of microorganisms can be met when the water has been treated with a suitable disinfectant.

To help foster this idea, the Current Good Manufacturing Practice 21 CFR 113 states in section 113.60 (b) "Container cooling water shall be chlorinated or otherwise sanitized as necessary for cooling canals and for recirculated water supplies. There should be a measurable residual of the sanitizer employed at the water discharge point of the container cooler."

It is obvious that a transient microleak in a container has no significance unless bacteria enter the container. Thus it is important that the cooling water contacting the container be of good quality. This correlation between good cooling water quality and prevention of post-processing leaker spoilage has been known for many years. As an example, Blackwood and Kalber (*2*) in 1943 filled size 307 × 409 cans with peas, sugar and water and subjected the containers to cooling waters of different qualities. The results of their study are shown in Table 1.

TABLE 1. *Effect of cooling water quality on post-process spoilage*[a].

Cooling water	Potable	Contaminated
No. bacteria per ml cooling water	3	700,000
No. cans tested	500	500
Cans spoiled	0	4

[a]From Blackwood and Kalber (*2*).

Put et al. (*13*), in 1972, found that reinfection of containers could be minimized if cooling water had less than 100 bacteria/ml, and if the number of bacteria in the water on the double seam at the end of container handling was less than 10,000/ml. Their surveys of 11 canneries in the Benelux from 1967-1969 showed that proper chlorination of can cooling waters assisted in minimizing the number of bacteria in the double seam water at the end of container handling. Graves et al. (*8*), in 1977, examined can cooling waters from 30 midwestern U.S. canneries during the 1974-75 canning season. They found that as residual chlorine increased total aerobic plate counts decreased, and that the incidence of coliforms was related to the total aerobic plate count; when the total count increased, coliforms were detected more frequently. Odlaug and Pflug (*12*) visited 17 cannery plants in Minnesota and Wisconsin in 1975 and 1976. They found the total aerobic count to be related to the hypochlorous acid concentration and water temperature. They also found the spore count to be independent of the total aerobic count.

All of these investigators found that the application of a germicide, usually chlorine, was beneficial in obtaining good quality (low bacterial numbers) cooling water. We would like to discuss the effect of several germicides used in cooling water on various microorganisms.

EFFECT OF CHLORINE

Chlorination is one of the most widely used means of disinfecting waters. Chlorination of cooling waters is usually accomplished with the use of sodium or calcium hypochlorite or with gaseous chlorine. The actual compound used is not essential, as long as a sufficient amount is added to obtain the appropriate amount of free available chlorine to destroy the target organism. Table 2 shows some typical results obtained from exposing various test organisms to chlorine solutions in distilled or buffered water solutions. The vegetative bacteria are easily destroyed. The aerobic sporeformers require more severe treatment for destruction, with the thermophile *Bacillus stearothermophilus* being most resistant to destruction. The anaerobic spore formers are less resistant than the aerobic sporeformers, but more resistant to destruction than the vegetative bacteria.

Our data in Table 3 show that proteolytic strains of *Clostridium botulinum* are more resistant to destruction

than the non-proteolytic strains. Resistance of the proteolytic and non-proteolytic strains of type F strains tested are similar. *C. botulinum* spores are easier to destroy in chlorine solutions than are aerobic spore formers. Our results compare favorably with those of Dye and Mead (*7*), particularly considering the differences in pH and temperature.

The bacterial activity of chlorine is dependent on pH, being greater at lower pH values. This is because undissociated hypochlorous acid is the active agent in chlorine disinfectants. As temperature increases, there is an increase in activity. When organic matter is present, more chlorine is required to maintain the needed amount of free available chlorine. This chlorine demand must be met for chlorination to be effective. Thus for the most effective use of chlorination in the cooling water, excessive organic debris should be avoided, the pH should be kept near neutral and the water temperature should be temperate. Obviously if the water temperature gets too warm, it will not serve its intended purpose, that of cooling.

Chlorine dioxide has in recent years been used more frequently in water disinfection. Because of its reactivity, it must be prepared at the place of use. Table 4 shows the effect of chlorine dioxide on microorganisms. Conditions similar to those needed for chlorine are needed to destroy vegetative bacteria. Higher concentrations are needed to destroy sporeformers. Unlike chlorine, however, chlorine dioxide appears to be more effective in destroying aerobic spore formers, even *B. stearothermophilus*, than in destroying the anaerobic sporeformers.

The bactericidal activity of chlorine dioxide is not dependent upon pH, having approximately the same effectiveness between pH 6 and 10 (*15*). The activity is affected by temperature, becoming less as the temperature is decreased (*16*). Chlorine dioxide does not react as chlorine does with organic matter. It does not react with ammonia and breaks down phenolic compounds. Thus it is more effectively used in water with high organic loads than is chlorine.

TABLE 3. *Resistance of* C. botulinum *types A, B, C, E, and F to chlorine solutions* (*10^4 spores/ml, pH 6.5 phosphate buffer, 25 C 4.5 ppm FAC*).

Type	No. of strains tested	Time to destroy 99.99% (min)
A	8	10.5
B (Proteolytic)	8	12.0
B (Non-Proteolytic)	1	5.5
C	2	3
E	4	6.0
F (Proteolytic)	1	8
F (Non-Proteolytic)	2	7

TABLE 2. *Effect of free available chlorine on various microorganisms.*

Organism	FAC (ppm)	pH	Temp. (C)	Exposure time (min)	Destruction results (%)	References
A. aerogenes	.01	7.0	20	5	99.8	(*16*)
S. aureus	.07	7.0	20	5	99.8	(*16*)
E. coli	.01	7.0	20	5	99.9	(*16*)
S. dysenteriae	.02	7.0	20	5	99.9	(*16*)
S. paratyphi B	.02	7.0	20	5	99.9	(*16*)
B. cereus	100	8.0	21	5	99	(*6*)
B. subtilis	100	8.0	21	60	99	(*6*)
B. macerans	7.5	6.5	25	8	99.99	(*17*)
B. coagulans	5	6.8	20	27	90	(*11*)
B. stearothermophilus	200	6.5	25	9	99.99	(*17*)
PA 3679	6.5	6.5	25	7	99.9	(*17*)
C. perfringens 6719	5	8.3	10	60	no destruction	(*7*)
C. histolyticum 10	5	8.3	10	10	90	(*7*)
C. tertium	5	8.3	10	20	99.9	(*7*)
C. bifermentans	5	8.3	10	20	99.99	(*7*)
C. sporogenes 532	5	8.3	10	35	99.9	(*7*)

TABLE 4. *Effect of chlorine dioxide on various microorganisms.*

Organism	FAC (ppm)	pH	Temp (C)	Exposure time (min)	Destruction results (%)	References
E. coli	.25	6.5	20	41 sec.	99	(*1*)
A. aerogenes	.01	7.0	20	5	99.8	(*1*)
S. aureus	.12	7.0	20	5	99.99	(*16*)
S. dysenteriae	.01	7.0	20	5	99.9	(*16*)
S. paratyphi B	.03	7.0	20	5	99.9	(*16*)
B. subtilis	1.0	7.0	20	10	99.9	(*14*)
B. mesentericus	2.0	7.0	20	10	99.9	(*14*)
B. macerans	20	6.5	25	11	99.99	(*17*)
B. stearothermophilus	25	6.5	25	12	99.99	(*17*)
P.A. 3679	100	6.5	25	3	99.9	(*17*)
C. botulinum 115B	125	6.5	25	14	99.9	Ito and Seeger unpublished
C. botulinum 62A	130	6.7	25	13	99.9	Ito and Seeger unpublished

EFFECT OF IODOPHORS

Iodophors are a complex or combination of iodine and certain surface active agents which slowly liberate or release free iodine when diluted with water. These compounds retain the beneficial germicidal activity of iodine and have lost some of the undesirable properties, such as their staining and allergenic properties.

Data in Table 5 show the effect of iodophors on various microorganisms. Iodophors, because of their various formulations, have different effects on microorganisms. The results listed are representative data. When more than one iodophor was tested, attempts were made to list only the severest condition. Iodophors are effective in destroying vegetative bacteria and yeasts. They are not very effective against either aerobic or anaerobic spore formers. In Table 6 are results of some of our studies on the resistance of *C. botulinum* spores to different iodophors. High concentrations of iodophors are required to get population reductions in a relative short time.

The bactericidal agent is free elemental iodine, thus the more acid the solution, the more iodine that is present. Thus these products are most effective in the acid pH ranges. To assure the acidity of the product, most iodophors are formulated with phosphoric acid. The activity of iodophors are not as affected by lower temperature as is chlorine. Iodine is also less reactive than chlorine and does not form a haloamine with nitrogenous compounds. Additionally the surface active agents assist in wetting and penetration through oily films.

EFFECT OF OZONE

Ozone is a gas with very strong oxidizing properties. It is unstable and is therefore generated on site. It has been used in Europe for disinfecting water for some time. Table 7 gives data showing the effect of ozone on microorganisms. The vegetative bacteria are readily destroyed. The sporeformers are more resistant to destruction with the aerobic sporeformers being more easily destroyed than the anaerobic spore formers.

The germicidal capability of ozone appears to be less affected by pH and temperature than that of chlorine. It is extremely corrosive and is poisonous in strong concentrations in the air. Ozone disappears rapidly from the water and its solubility is dependent upon temperature and pressure. Because of its high reactivity, the organic load of the waters affects the amount of ozone entering the water. Thus waters with low organic loads are preferred.

CONCLUSION

A number of germicides are available for disinfecting container cooling water. Each has its advantage as well as disadvantages. In practical application, the most effective use of the germicide will be realized when it is applied with full knowledge of its strength and weaknesses. The systems and procedures must be

TABLE 5. *Effect of iodophors on various microorganisms.*

Organism	Conc. (ppm)	pH	Temp (C)	Exposure time (min)	Destruction results (%)	References
S. aureus	< 10	2.5	20	30 sec.	99.9999	*(10)*
S. lactis	6	5.0	25	60 sec.	killed	*(9)*
L. plantarum	6	5.0	25	2	killed	*(9)*
P. cerevisiae	6	5.0	25	2	killed	*(9)*
Yeasts	25	4.4	15	8 sec.	90	*(3)*
B. subtilis	100	2.3	21	240	99	*(6)*
B. cereus	100	2.3	21	12	99	*(6)*
B. macerans	500	6.5	25	> 60	99.99	*(17)*
B. stearothermophilus	500	6.5	25	> 60	99.99	*(17)*
P.A. 3679	500	6.5	25	> 60	99.99	*(17)*

TABLE 6. *Resistance of* C. botulinum *types A, B, E to Iodophors.*

Iodophor	Type A		Type B		Type E	
	Conc. (ppm)	Min.	Conc. (ppm)	Min.	Conc. (ppm)	Min.
Butoxypolypropoxy polyethoxy ethanol-iodine complex	1640[a]	26	1640[a]	23	100[b]	6
Nonylphenoxy polyethoxy ethanol-iodine complex	1500[a]	20	1500[a]	30	100[b]	5.5
Polyethoxy polypropoxy polyethoxy ethanol-iodine complex	1700[a]	22	1540[a]	24	—	—

[a]45 C, pH 2.0.
[b]25 C, pH 2.5.

TABLE 7. *Effect of ozone on various microorganisms.*

Organism	Conc. (ppm)	pH	Temp (C)	Exposure time (min)	Destruction results	References
S. aureus	0.5		25	15 sec.	Destruction	(5)
S. typhimurium	0.5		25	15 sec.	Destruction	(5)
E. coli	0.5		25	15 sec.	Destruction	(5)
S. flexneri	0.5		25	15 sec.	Destruction	(5)
B. cereus	2.29		28	5	Destruction	(4)
B. megaterium	2.29		28	5	Destruction	(4)
B. macerans	2.0	6.5	25	1.7	99.9%	(17)
B. stearothermophilus	3.5	6.5	25	9	99.9%	(17)
C. perfringens	0.25	6.0	24	15	Destruction	(18)
P.A. 3679	5	3.5	25	9	99.9%	(17)
C. botulinum 62A	6	6.5	25	2	99.9%	Ito and Seeger, unpublished
C. botulinum 213B	5	6.5	25	2	99.9%	Ito and Seeger, unpublished

designed to be used under the best conditions possible.

One-use waters, usually city or well water, are usually used for cooling water purposes. These are usually of good microbiological quality and low in organic matter. However, germicides should still be applied to assure proper disinfection at a point of use. To conserve water and energy, many canneries are using recirculated water systems. In these systems, careful attention must be given to insure adequate germicidal application. Recirculation systems are prone to build-up of organic material as well as microorganisms, and thus care must be taken to prevent or control such build-ups.

A regular schedule of monitoring the applied germicides at point of application, and at critical areas in the system, should be established. The aim should be to obtain a detectable level of the germicide at a point immediately after the containers have been through the cooling cycle.

The purpose of adding germicides to cooling waters is to reduce the microbial load to low numbers. By keeping the water at a germicide level sufficient to destroy vegetative bacteria in the contact time available, the canner is able to meet this goal, particularly in one-use water applications. In recirculated water systems, periodic high level application of germicides is needed to remove microorganism build up. Thus with knowledge of the resistance of microorganisms to germicides, an effective operating system can be designed to obtain low numbers of microorganisms in container cooling waters.

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Mechanism of Microbiological Leaker Spoilage of Canned Foods: Biophysical Aspects

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ABSTRACT

The three main factors in spoilage resulting from post-processing can handling operations are: (*a*) condition of the can double-seams, (*b*) presence of bacterial contamination in cooling water or on wet can runways and (*c*) can abuse due to poor operation or adjustment of the filled can handling equipment. The mechanism of bacterial reinfection and the way it is influenced by deviations in can construction or can handling procedures is discussed. Methods of preventing bacterial reinfection at the most critical points in the canning operations are given as well as certain guiding principles for canning practice. In addition, some remarks on physical aspects of reinfection of glass-packed foods are made briefly.

Nicolas Appert, 1750-1841, is generally recognized as the father of canning and thermal processing. In 1809, Appert was given 12,000 francs for publication of his secrets on food packaging and preservation. In 1810, his secrets were published in a booklet entitled: "L'Art de Conserver, Pendant Plusieurs Années, toutes les substances animales et végétales" (*3,4*). This means: "Art of preserving animal and vegetable substances".

Louis Pasteur's (1822-1895) work, however, formed the foundation of the science of microbiology as well as of the science of heat preservation of packaged foods. Pasteur (*24*) demolished the false temple and false philosophy of spontaneous generation. On that account, the work of Pasteur served as a basis for transformation of canning from an art into an applied science.

Pasteur showed how to sterilize a liquid by hot air and how to keep it sterile. The original glass flasks with which this sterilization method was demonstrated bear some resemblance to the structure of the double seam.

The science and technology of canning at the moment, deals mainly with: (*a*) applied microbiology, (*b*) process technology, (*c*) process equipment and (*d*) process engineering.

In his booklet, Nicolas Appert (*3*) defined the preservation of foods by canning on two main principles: (*a*) the food or feed is conditioned in a container which is *sealed* and therefore "impenetrable" to liquids, gasses and micro-organisms; (*b*) the sealed container is subjected to *heat* or any other *treatment* (radiation) so as to inactivate or destroy enzymes, microorganisms and their toxins (except most mycotoxins) that cause spoilage or toxicity of the food product.

Sealing, the first principle of Appert's art of preservation can be realized by corking of a flask (*3*), capping of a glass jar (*8*), seaming or soldering of a metal container (*16*), sealing of a flexible pouch (*1,25*).

The first artistic flask corking machine (Fig. 1) was designed by N. Appert (*3,4*) who explained that: "Le parfait bouchage est de la plus grande importance pour obtenir la conservation des substances alimentaires." This means: "to obtain preservation of the food product, a perfect corking is of the highest importance."

Since that time, many other glass jar closures have been developed. The "crown" cap closing of narrow-neck bottles, for instance, is widely used for beer, mineral waters and soft drinks. Besides, a great variety of heat-processed foods are packed in wide-neck glass containers hermetically sealed with metal closures of lacquered tinplate or aluminum.

The basis of all glass closures is a metal cap that is firmly held against a flowed-in gasket or compound which seats on or around the rim of the glass.

Wide-neck glass jar closures can be separated into three groups according to whether the cap is held in position by mechanical means (Twist-Off cap and screw cap closures), vacuum retention (White cap), mechanical and vacuum retention (Omnia, Pano and Pano T). The latter group of closures is predominantly used in industrial food packaging in glass containers in The Netherlands.

The problem of leaker spoilage of canned foods had already been recognized by Appert. In the 4th edition of his booklet on "L'art de conserver..." (*4*), Appert introduced in chapter IV the following statements on guarantees of his preserved food products: "All the substances, destined particularly for voyages at sea and for long trips, must be entrusted aboard the ships, under the surveillance of a person who could, by his care, guarantee them from all accidents of travel, notably from being thrown upside down, mistreated or placed in humid places. These precautions are the most urgent, inasmuch as the least shock that they can receive will produce damage in the tins and breakage of the glass vases. In the instance where, upon returning, the state of the tins will indicate that these precautions have not been observed, there will not be any replacement. In the damaged tins, that is to say those that are convex or bulging, which will be set aside to be reported upon return, it is possible to find one or more out of 25 which

will need to be thrown into the ocean because of a bad odor coming from a small opening, either in the closure or in the body of the tin. This is a recognized truth by more than 15 years of experience. However, this loss is a very small thing compared to those which one encountered with stewing, salting and so many other foods on which the furnisher has no recountability".

In industrial practice now, reinfection of canned and bottled foods in frequently observed in apparently well-constructed cans and well-shaped glass jars with high quality seams and closures which cannot be shown to leak by any of the traditional test methods.

As a consequence of reinfection of canned foods by non-pathogenic and pathogenic microorganisms, economic losses and food poisoning may occur (*17,24*).

In this paper, a contribution on biophysical factors of microbiological leaker spoilage of canned and bottled foods is given, and, in addition, some practical principles of its prevention.

MATERIALS AND METHODS

Test organism

Enterobacter aerogenes code ENT 2.3, *Citrobacter intermedius* code ENT 3.1, *Hafnia alvei* code ENT 2.4.

Preparation of biotest inoculum

See scheme (Table 1). The citrate broth used consists of (g/1): sodium citrate, 2.5; $Na(NH_4)HPO_4 \cdot 4H_2O$, 1.5; $MgSO_4 \cdot 7H_2O$, 0.2; K_2HPO_4, 1; NaCl, 5; in distilled water; pH, 6.8.

Biotest of cans under static postcooling conditions

A large sample of cans, 76 × 76 mm, with both ends seamed in place and with a small filling hole in the center of one end, were leak-tested by internally pressurizing them with air at 1 bar internal pressure and then immersing them in water. In this manner, experimental cans were selected which exhibited constant leaks of such a size that ca. 0.01 ml of air at NTP escaped in 15 sec. The cans so selected were 1/3 filled with citrate broth in distilled water, pH 6.8. This nutritively sparse medium was chosen as it was unlikely to block capillary pathways with precipitated protein.

The filled cans were sterilized and cooled in sterile water. The cooling water in the outer part of the double seam was removed by vaporization in a vacuum system and replaced with water or citrate broth inoculated with various numbers of the test organism. The exterior double seam was maintained in contact with the infected medium at constant temperature for a predetermined period before being washed off with tap water. A proportion of cans were then dried, others being left wet, according to the experimental design. All cans were subsequently allowed to stand at constant temperature for various times before their contents being membrane filtered (Millipore; Benelux N.V., Heliotropenlaan 10, B-1030 Brussels, Belgium) to count the infiltrating bacteria.

Biotest of cans under dynamic postcooling conditions

Electro magnetic hammer abuse. This modified biotest consisted of filling cans of 73 × 57.5 mm with citrate broth of ca. 70 C, to which 0.1% of glucose was added. The filled cans that have a head space of ca. 8 mm height were sealed on a Metal Box (MB) double seamer. The seam setting was varied depending on the type of seam structure to be tested. Factors of the can structure to be tested were bodyhook, seam tightness, seam thickness, wrinkle grade, compound type, compound weight, compound lining, side seam and -lap construction, tinplate

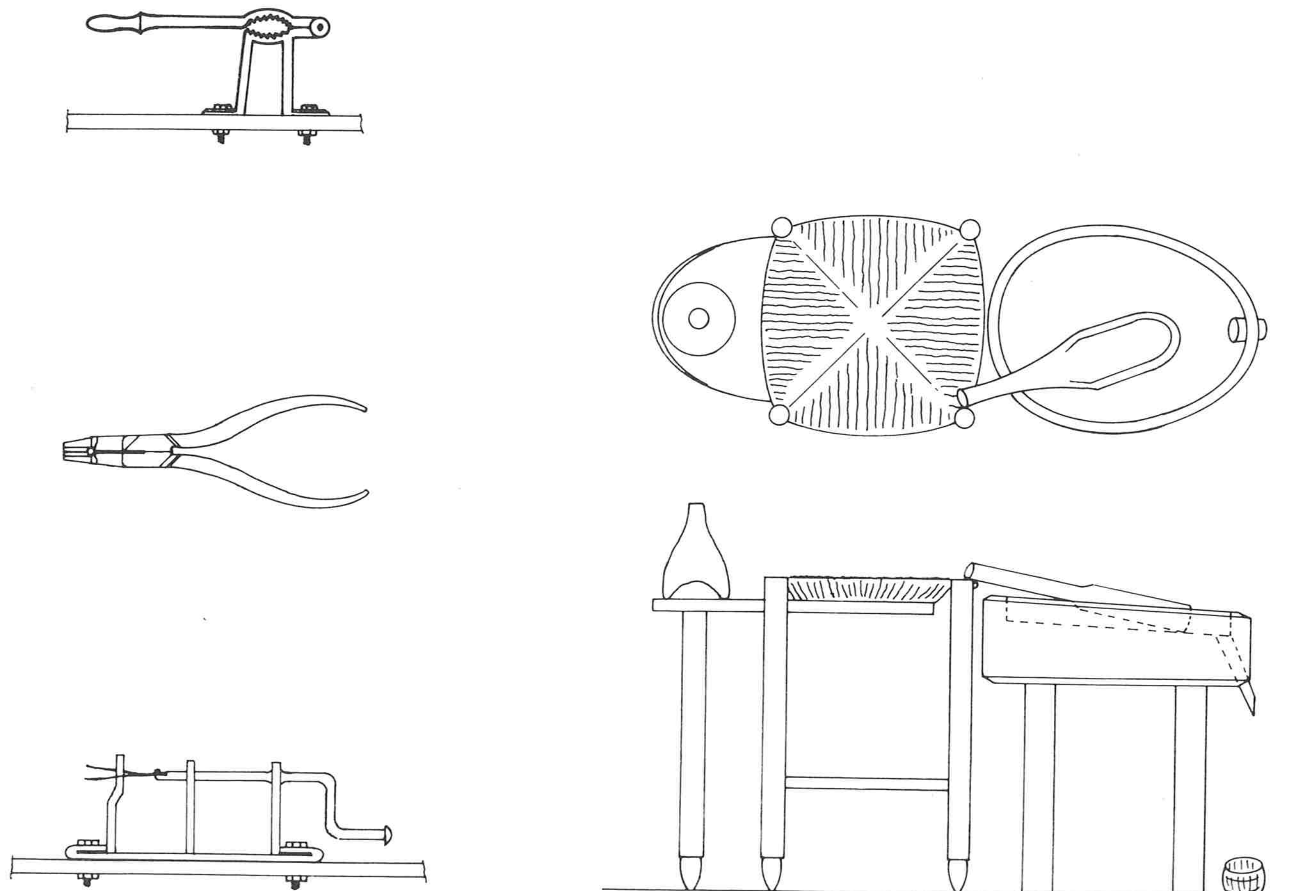


Figure 1. *Design of a bottle corking machine* (*Appert, 1813*).

hardness and thickness of the body and ends, inside lacquering and top-end profiles.

The closed cans were processed at 121 C for 20 min and cooled under pressure to ca. 25 C, whereafter the wet cans were "post-cooled" during 4 h at 25 C in water containing ca. 10^8/ml of viable cells of *C. intermedius*. The wet cans were subsequently subjected to a standardized mechanical impact (*a*) on the side seam adjacent to the double seam, or (*b*) on the double seam at the extension of the side seam, by means of an electromagnetic hammer. The apparatus, manufactured by Autelca A. G., Grümlingen, Berne, Switzerland, is fitted with a number of interchangeable hammers shaped like segments of double seams of various diameters. Depending on the can diameter, and can content weight, one to three different hammer settings were tested. The hammer abuse is given at 25 C for 0.1 sec. The depth of the dent (ca. 0.45, 0.70, 1.05 mm, respectively) as a result of the hammer-blow, on the side seam, corresponds to what can happen in normal industrial food canning processes. Its depth being dependent on the body tinplate thickness used and to a lesser extent on the can diameter and the can height (Tables 2 and 3).

The abuse was followed by rinsing the cans with tap water and incubation for 10-14 days at 25 C. Checks on swells were made every 24 or 48 h. Leaking cans become evident as they swell during incubation, due to the microbial evolution of gas (from glucose) within the can.

Free-falling hammer abuse. The biotest procedure was the same as just described. However, the mechanical abuse was delivered by a free-falling hammer of 300 or 600 g. A drop height of 20 or 50 mm was used. The number of blows delivered on the crossover of each can was a maximum of 200.

Biotest of glass jars

Glass jars (diameter of 63 mm; volume of ca. 200 ml) were filled with citrate broth of ca. 75 C, and closed. The head space of the jars was constant (ca. 8 mm). The initial vacuum was regulated on ca. 30 and ca. 60 cm of Hg at 20 C. Depending on the tightness factor to be tested, enclosures were introduced: (*a*) nylon thread, diameter of 0.1 or 0.15 mm; (*b*) cotton fiber diameter of 0.4 mm; silk fiber diameter of 0.3 or 0.4 mm, or (*c*) vegetable filaments including spinach, red cabbage or curly cabbage. The jars were sterilized at 115 C for 60 min and subsequently cooled in a sodium fluorescein solution of 4% in tap water, which concentration is not toxic for the biotest strain. The cooling process was followed by thoroughly rinsing the jars with tap water, whereafter fluorescein infiltration was measured colorimetrically.

The jars were then placed for 4-14 h upside down in the postcooling contact medium containing ca. 10^8/ml of viable cells of *E. aerogenes*.

The wet jars were rinsed and incubated, upright or upside down, for 28 days at 25 C. Checks on bacterial reinfection were made at 2-day intervals.

TABLE 2. *The influence of can size on mechanical abuse.*

Can size (mm)	Plate thickness, mm		Depth of dent, mm — Hammer-blow force	
	Body	End	2	3
76 × 57.5	0.18	0.22	0.60	1.10
	0.21	0.25	0.50	1.05
76 × 100	0.19	0.24	0.60	0.95
	0.21	0.24	0.50	0.90
76 × 126	0.19	0.23	0.65	1.10
	0.22	0.23	0.40	1.00
102 × 118.5	0.22	0.26	0.60	0.90
	0.25	0.28	0.50	0.80

TABLE 3. *Rough handling in canning practice.*

Can size (mm)	% of cans with dents of mm			
	< 0.40	0.40-0.60	0.65-1.10	> 1.10
76 × 57.5	30	65	5	1
76 × 100	35	45	15	5
76 × 126	30	55	10	5
102 × 118.5	5	30	30	35

The manganese ion test

The manganese ion test involves detection of traces of Mn^{2+} passing through the seams, the test being made simultaneously with a biotest. In this, processed cans, after being mechanically abused, were cooled in a 7% solution of $MnSO_4$ infected with 10^7 viable bacteria/ml. The bacteria used were insensitive to Mn. After cooling, thoroughly washing and drying, the contents of each can were membrane-filtered to determine the number of bacteria which have entered; the filtrate from each can being used to estimate, by atomic adsorption analysis, the amount of $MnSO_4$ which has entered each can. The manganese concentration is directly proportional to the water ingress (*6*).

The manganese ion was chosen as the most suitable tracer because, owing to its place in the electrochemical series, it is not plated out on tin or steel. Furthermore, it is not a probable contaminant from components of the can or from extraneous sources.

The helium gas leakage test

The helium test involves use of a mass spectrometer (Series 400 Centronic, 20th Century Electronics Ltd.) tuned to detect helium leakage rates in the range of 10^{-10}-10^{-3} ml/sec at normal temperature and pressure (NTP). The principle of this technique is illustrated in Fig. 2. The test can was evacuated continuously by a 50 l/min, 2-stage, rotary vacuum pump (Speedivac 2SC 50B; Edwards, Sussex). As soon as the pressure had fallen to < 10^{-1} Torr, sampling valve B was opened and helium was sprayed around the outside of the can. If any helium penetrates the seams, the mass spectrometer responds proportionately.

TABLE 1. *Preparation of the biotest inoculum.*

Step No.	Medium		Incubation Time (h)	Incubation Temp (C)	Storage Time (year)	Storage Temp (C)
1	Glucose horse-serum lyophilized 10-fold	ampule			5-10	20
2	Nutrient broth	tube	16	32		
3	Nutrient agar	streak plate	16	32		
4	Nutrient broth one colony	tube	16	32		
4a	Test purity and taxonomic properties					
5	Nutrient agar 25 fold	byoux	16	32	0.5	5
6	Nutrient broth pre-inoculum	tube	8	32		
6a	Test purity					
6b[a]	Glucose horse-serum lyophilization 10 fold	ampule			5-10	20
7	Citrate broth 100 ml	flask	16	32		
8	Citrate broth 500 ml n×	flask	8	32		
9	Citrate broth inoculated 1 : 20 post-cooling biotest	bath ca. 100 l	4	25-30		

[a]Facultative.

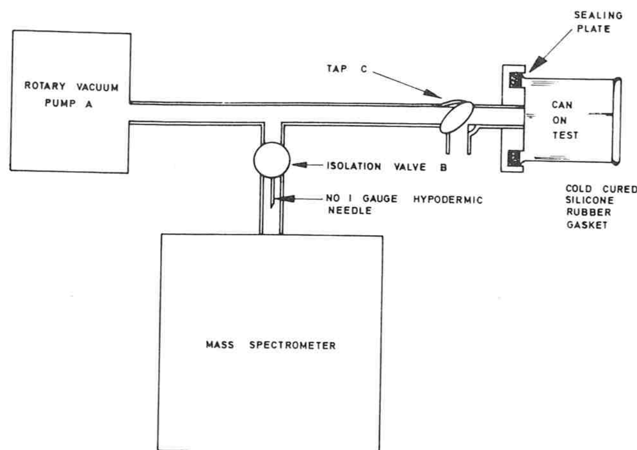


Figure 2. *Outline of the helium tuned mass spectrometer technique for determining the gas leakage rate of cans (Perigo, personal communication).*

The mass spectrometer, although expensive, yields rapid accurate results, a single determination taking ca. 30 sec. Besides, the test is a non-destructive one, hence it can be combined with a biotest or any other can tightness investigation.

Fat resistance tests of compounds for ends

Striptest

The compounds to be tested were lined up on small strips (10 × 66 mm) of tinplate, which were lacquered or plain. The compound film thickness was constant and comparable to the film thickness in the double seam.

The strips were covered with a layer of fat, e.g. lard, beef suet, chicken fat, crock-vitol (a poly-unsaturated fatty acids containing diet margarine), put in cans, sterilized in steam of 120 C for 20 min and cooled to 20-25 C.

Then a hollow tube with an external diameter of 3 mm was moved under a constant load, a constant angle of 45° and a constant speed of 0.5 cm/sec over the horizontally placed strips. The number of holes, made by the moving tube were counted and their length was measured.

A fat-resistant compound will show a large number of small holes, while low fat resistance will give rise to a low number of long slit-shaped holes.

Biotest for fatty packs

The biotest was carried out as described above. However, in addition to this test scheme, a fat-factor was introduced. For this purpose, a thin layer of fat (crock-vitol) was brushed on the compound of the ends 24 h before biotesting the cans.

RESULTS AND DISCUSSION

Under static postcooling conditions, the frequency of reinfection was primarily influenced by the number of bacteria in the water trapped in the outer part of the double seam (Table 4) and the time during which the seam remained wet (Table 5).

In addition, the frequency of reinfection was enhanced secondarily as a result of the motility of the infiltrating bacteria (Table 6). At the same time, the motility as well as the frequency of reinfection were decreased as a consequence of an increasing viscosity of the infected liquid in the outer part of the double seam (Table 7).

Besides, it was observed that the cooling water infiltration rate as a function of the number of bacteria passing continuously through the double seam was much lower than was calculated.

TABLE 4. *The influence of the number of bacteria on the frequency of re-infection.*

No. of bacteria/ml of water[a]	% of cans re-infected[b]
10^2	2
10^3	8
10^4	18
10^5	30
10^6	48
10^7	62
10^8	70
10^9	88

[a]*Enterobacter aerogenes.*
[b]Static postcooling conditions.

TABLE 5. *The influence of contact time on the frequency of re-infection.*

Contact-time at 17.5 C	% of cans re-infected, when seam initially filled with: Sterile water	Contaminated water[a]
3 sec	2	
15 sec	10	
30 sec	16	
1 min	20	
5 min	24	55
10 min	28	58
30 min	35	62
1 h	50	58
2 h	58	64
3 h	60	66
4 h	62	64

[a]10^9/ml. *E. aerogenes*, static post-cooling conditions.

A capillary path length of ca. 2 mm through the double seam, through which at 1 bar of pressure ca. 0.01 ml of air at NTP can pass, corresponds with a pore size of 0.010-0.015 mm, through which could have passed many more bacteria of 0.5 × 1.0 μm, in a much shorter time than actually observed, referring to an initial number of ca. 10^8 bacteria per ml (Table 8).

We therefore concluded that the leakage path through the double seam was not a straight one, hence it has a certain capacity to filter out microorganisms passing through it (*6,18*).

In canning practice, a permanent leak path through the double seam may exist due to: (*a*) an improper seam construction (Table 9), (*b*) use of non-leak resistant compounds (Table 10 and Fig. 3) and (*c*) improper side-seam soldering and side-seam tightness (Fig. 4).

Under dynamic postcooling conditions, the bacterial reinfection frequency is enhanced as a consequence of physical factors and mechanical impacts. The most important physical factors are: constant pressure differences inside and outside the can (Table 11), while at the same time sudden pressure changes during sterilization and cooling may induce reversible as well as permanent deformations of the double seam and hence an increase of the leaker infection frequency (Table 12).

Reversible and permanent seam deformations may also be caused by mechanical impacts. Perigo (personal communication) observed that the effect of light and repeated blows on the seam is cummulative and may lead

TABLE 6. *The influence of bacterial motility and contact-time on the frequency of re-infection.*[a, b]

Contact time	% of cans containing infiltrated bacteria in the range of < 1	10	10^2	10^3	10^4	10^5	10^6	10^7/can
	Aerobacter aerogenes (motile)							
At 12.5 C								
30 min	65	5	15	15	0	0	0	0
1 day	53	10	10	20	7	0	0	0
2	28	2	18	50	2	0	0	0
3	23	0	10	35	5	20	7	0
4	18	0	8	30	17	5	17	5
5	15	0	3	5	22	10	15	30
6	10	0	0	0	20	16	22	42
	Hafnia alvei (non-motile)							
At 7.5 C								
30 min	94	4	2	0	0	0	0	0
1 day	89	5	4	2	0	0	0	0
2	84	7	7	2	0	0	0	0
3	81	13	4	2	0	0	0	0
4	69	16	10	5	0	0	0	0
5	62	14	14	10	0	0	0	0
6	51	6	20	21	2	0	0	0

[a]Post cooling water contains 5×10^8/ml of bacteria in a static position.
[b]Determined by membrane filtration.

TABLE 7. *The influence of viscosity and bacterial motility on the frequency of re-infection.*

Viscosity[a] (Millipoise at 25 C)	% of cans re-infected[b] with *E. aerogenes* (motile)	*Hafnia alvei* (non-motile)
0.9	70	22
1.1	68	12
3.0	66	6
7.5	56	4
18	24	2
26	6	1
42	2	< 1

[a]Viscosity factor: Difco Agar, in post-cooling water.
[b]Under static post-cooling conditions.

TABLE 8. *Simultaneous determinations of water and microbial can leakage.*

	Leakage rate × 10^{-7} ml of water: Chemical[a]	Microbial[b]
% of leak cans	100	25-50
Leakage rate		
Min.	2×10^3	6
Max.	1.7×10^6	3.5×10^5

[a]$MnSO_4$; Bashford et al., 1962.
[b]Standard biotest; membrane filtration.

TABLE 9. *The influence of the double seam structure on the frequency of re-infection.*

Test series[a]	Size (mm) of Bodyhook, a	Overlap, b	Seam tightness, c	% of cans re-infected
1	1.38-1.45[b]	0.85-1.05	0[c]	28
			1	82
			2	98
2	1.75-1.90	1.10-1.20	0	7
			1	38
			2	90
3	1.90-2.10	1.35-1.50	0	5
			1	11
			2	35
4	2.15-2.20	1.45-1.55	0	0.5
			1	2
			2	8

[a]Standard biotest.
[b]Underlined values are sub-standard.
[c]0 = tight, 1 = normal, 2 = loose.

to an incidence of reinfection similar to that resulting from a singular more violent blow (Table 13).

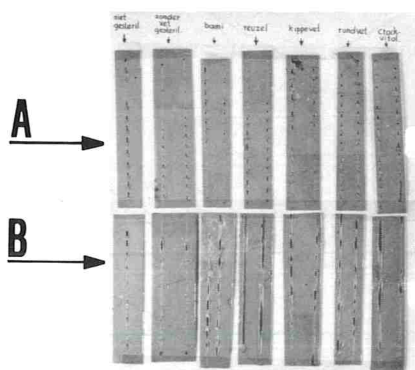
In canning practice, a seamer can operate at speeds up to and above 600 cans per min. Seaming and can transport at such speeds, which are progressively introduced in modern canneries, may give rise to increasing risks of seam damage, resulting in transient and permanent can leakage (*7,12,26*).

TABLE 10. *The influence of the type of lining compound on the frequency of re-infection.*

Seam tightness[a]	% of cans re-infected[c]: Compound 1 A[b]	Compound 1 F[b]	Compound 2 A	Compound 2 F	Compound 3 A	Compound 3 F
Tight	< 0.5	< 0.5	< 0.5	2	27	< 0.5
Normal	2	< 0.5	4	20	38	0.5
Fairly loose	8	1	10	34	58	1
Loose	12	4	16	72	90	3

[a]Body hook length and overlap are normal.
[b]A = aqueous, F = fatty.
[c]Standard biotest; underlined values are sub-standard.

STRIPTEST

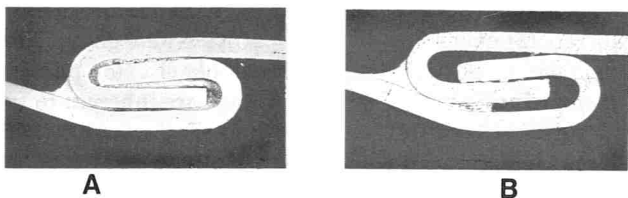


FAT RESISTANCE & ADHESIVITY

A: NORMAL

B: SUB STANDARD

Figure 3. *Strip test. A, compound adhesively normal. B, compound adhesivity substandard. Fat factors: 1, none; 2, none after sterilization; 3, fried rice; 4, lard; 5, chicken fat; 6, beef suet; 7, diet fat.*



A B

SIDE SEAM CONSTRUCTION & SOLDERING

A NORMAL B SUBSTANDARD

Figure 4. *Cross-section of a side seam. A, normal; B, improper soldering.*

A transient leak in some respects is similar to that of a flexible tube which, when subjected to slight compression, may suddenly eject the liquid which it contains (*24*). However, permanent leaks that are the result of physical and mechanical seam deformations, are often not detectable by traditional test methods (*11,19*).

Perigo and co-workers (personal communications) therefore developed a helium gas mass spectrometer leakage test method (Fig. 2). The helium test did show a much greater sensitivity to can leakage than obtained by the remaining physical (air leakage test), chemical (manganese ion test) and microbiological (biotest) methods.

Since that time (1965) this test principle has been further developed. Helium gas mass spectrometer leak detection systems are widely used now in can production quality examinations. The helium test, although more sensitive, did show a good correlation with the biotest.

From the results obtained, it could be concluded: (*a*)

TABLE 11. *The influence of constant pressure differences on the frequency of re-infection.*

No. of bacteria/ml post-cooling water[a]	% of cans re-infected		
	Pressure[b] 40	None 0	Vacuum[b] 40
10^9	22,5	24	54
10^7	18	20	37
10^5	5	6	16
10^3	1	1	2

[a]*E. aerogenes* in standard biotest.
[b]cm Hg 20 C; underlined values are sub-standard.

TABLE 12. *The influence of headspace vacuum & sterilization conditions on the frequency of re-infection.*

Canning conditions 20 C		% of cans re-infected[a]	
		Pressure differences	
Head space (ml)	Vacuum (cm Hg)	Normal	Abrupt
0	0	2-12[bc]	15-44[c]
0-2	25	1- 8[b]	10-32[c]
20	25	< 1- 5	6-20[b,c]

[a]During sterilization and cooling; standard postcooling biotest.
[b]Reversible distortion of the double seam.
[c]Permanent distortion of the double seam.

The crossover region of the double seam is much more impact-sensitive and leak-prone than other seam areas (Fig. 5, 6 and 7). In this respect biotest results were 10-20% (crossover region) and < 1% (other seam areas). (*b*) As a result of an increasing number of light hammer-blows on the crossover region, the helium leak size, the helium leak frequency as well as the reinfection frequency and reinfection rate of a leak sensitive can are progressively increased (Fig. 8; Table 13). (*c*) It was shown that the seam barrier properties of lining compounds are very important (Fig. 9, 10 and Table 10). (*d*) In conclusion, a maximum helium leakage rate of

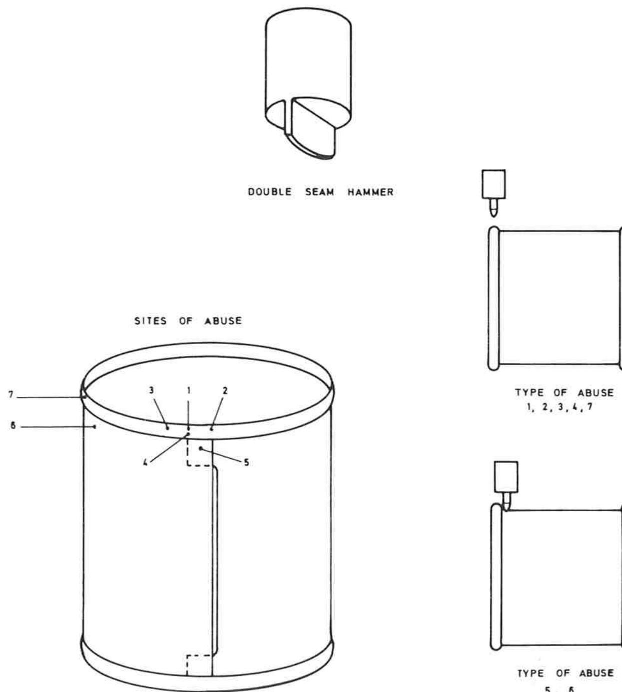


Figure 5. *The induction of can leakage by different types (7) of mechanical abuse (Perigo, personal communication).*

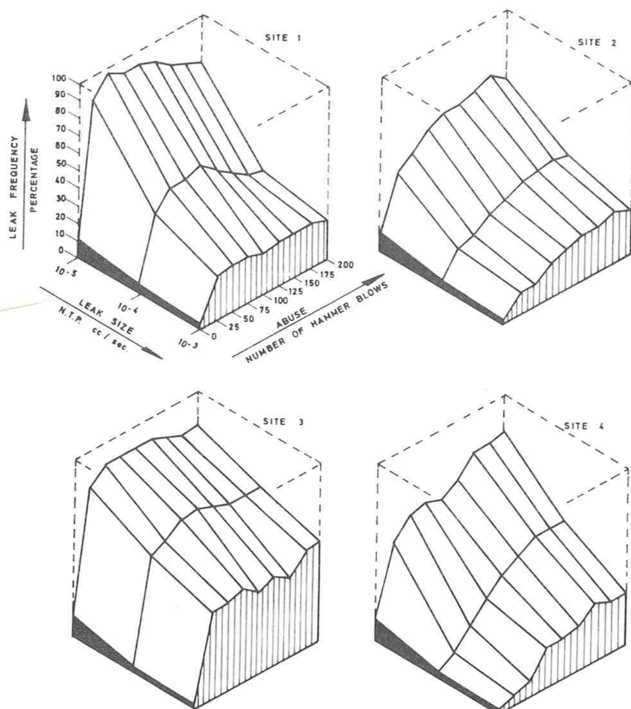


Figure 6. *The influence of the mechanical abuse site (1-4) on the helium gas leakage rate at NTP* = Normal Temperature and Pressure *(Perigo, personal communication).*

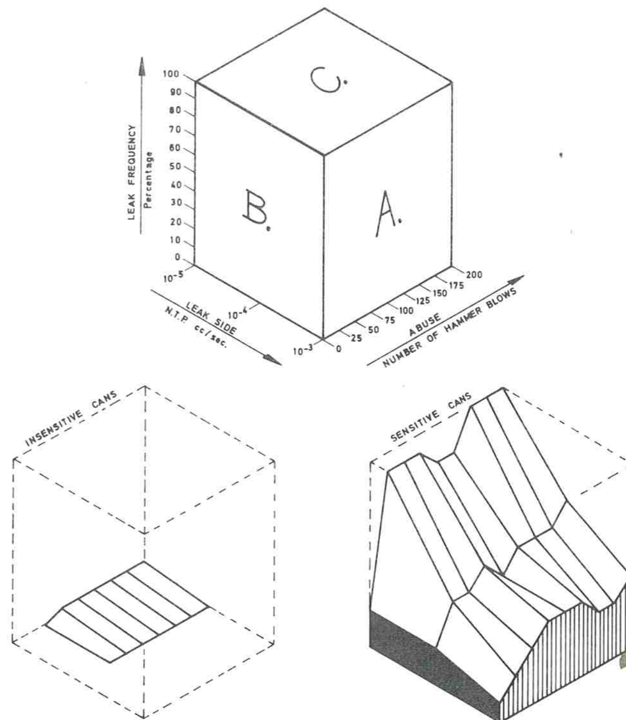


Figure 8. *The interpretation of helium gas leakage profiles of abused cans: abuse insensitive cans; abuse sensitive cans (Perigo, personal communication).*

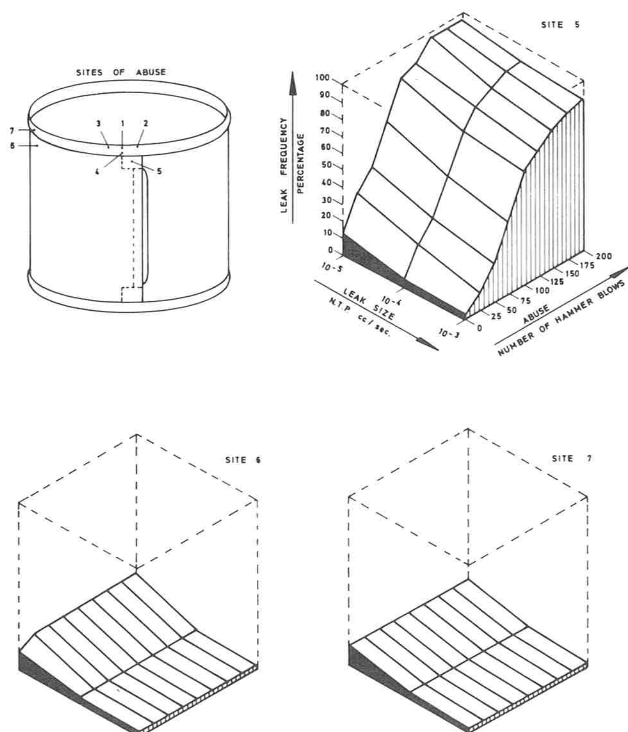


Figure 7. *The influence of the mechanical abuse site (5-7) on the helium gas leakage rate (Perigo, personal communication).*

5.10^{-3}-10^{-4} ml per sec at NTP, and a maximum tolerable number of microorganisms of 10^4 per ml of postcooling water trapped in the outer part of the double seam, could be derived (Fig. 11).

Heat preserved foods packed in sealed glass jars are more sensitive to reinfection than double seamed cans because (*a*) the leakage route through the closure is more direct, (*b*) enclosures of food particles and glass finish damage is difficult to avoid completely, (*c*) physical factors during processing and storage are inherent to the closure system and its tightness and (*d*) the sensitivity to mechanical impacts is relatively high (*8*).

In this respect, non-ventilating closures (Twist-Off, Table 14) are less sensitive to physical and mechanical factors than ventilating ones (Omnia, Table 15).

Insufficient temperature-pressure control inside and outside the glass jar during sterilization and cooling may lead to a too low initial vacuum, or loss of vacuum due to air leakage. Normal temperature-pressure diagrams during sterilization of some glass-packed food products are shown in Fig. 12, 13 and 14. Besides, failures of ventilating closures may be increased as a consequence of compound quality.

Depending on the type of compound; the type of foodpack, and its preservation process: (*a*) cooling water infiltration, (*b*) a sudden loss of vacuum (air leakage), (*c*) postcooling water infiltration (leaker infection) and (*d*) a slow loss of vacuum (air leakage) and multiplication of microorganisms through the closure may occur during subsequent storage (Table 15 and 16).

Glass packs which show gross leaks and rapid loss of vacuum are easily detected immediately after sterilization and cooling. However, a slow loss of vacuum (slow air leakage) is difficult to detect, as is a slow infiltration of microorganisms, caused by their motility and multiplication through a capillary path in the enclosed vegetable filaments. Such a leakage may not be detectable unless after a long lapse of time, the contents

TABLE 13. *The influence of the number of impacts and the number of bacteria on the frequency of re-infection.*

No. of hammerblows[a]	No. of bacteria/ml[b] post-cooling water					
	10^2	10^3	10^4	10^5	10^6	10^7
	% of cans re-infected					
0	0	0	1	1	6	8
25	0	1	3	8	16	24
50	0	2	5	12	32	42
100	0	4	10	22	44	62

[a]On the cross-over.
[b]*E. aerogenes*

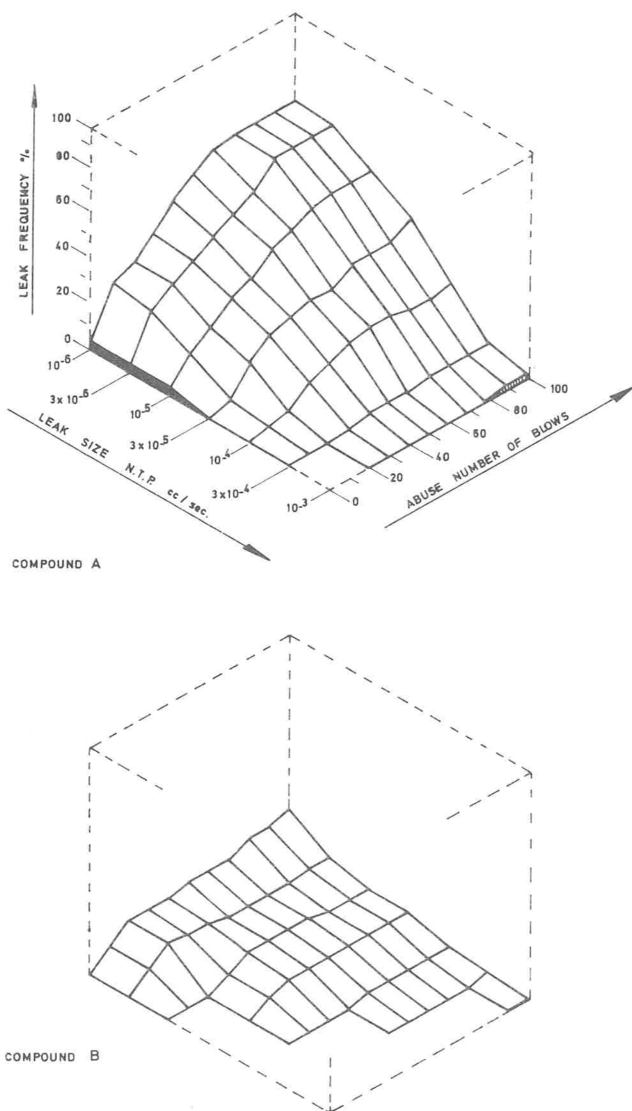


Figure 9. *The influence of the type of lining compound (A and B) and mechanical abuse on the helium gas leakage rate (Perigo, personal communication).*

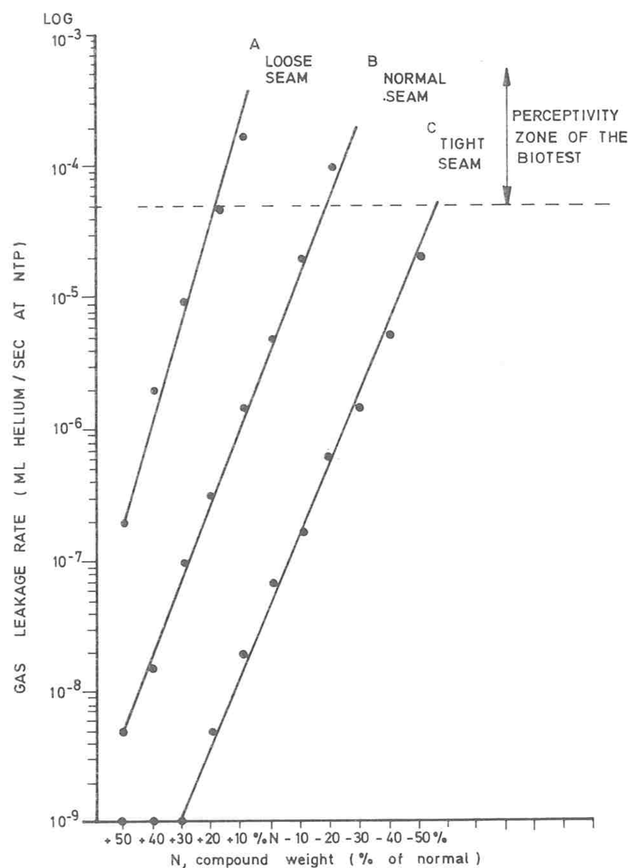


Figure 10. *The can lining compound film weight and the seam tightness compared with the helium gas leakage rate and the bacterial reinfection frequency. A, loose seam; B, normal seam; C, tight seam; ←→, perceptivity zone of the biotest (Put et al., 1972).*

TABLE 14. *Twist-off non-ventilating glass-jar closure leakage of A: water, B: air; C: bacteria.*

Enclosures	Cooling		Post-cooling & Incubation	
	A water (%)	B air (+ / −)	C Bacteria (%)	B Air (+ / −)
Non	< 0.4	−	< 0.4	−
Nylon, diam. 0.1 mm	< 0.4	−	4	−
Cotton, diam. 0.4 mm	< 0.4	−	2	− / ±

A, Fluorescein; B, Loss of vacuum; C, 30 d, 25 C.
Compound I; I vac; 30-40 cm Hg 20 C.

of the jar become spoiled (Table 17).

Leaker infection of heat-preserved canned and glass-packed foods can be prevented by: (*a*) a double seam, side seam and glass closure construction up to the standard requirements, (*b*) avoiding of enclosures on the glass rim, (*c*) a correctly controlled and carefully checked sterilization and cooling process (*2,13,14,15*), (*d*) disinfection of the cooling water by chlorination up to 2-3 μg free Cl per ml of water (*27*) which should result in a maximum bacterial count < 100 per ml, being furthermore in conformity with the chemical and bacteriological standards for drinking water (*10*), (*e*) the

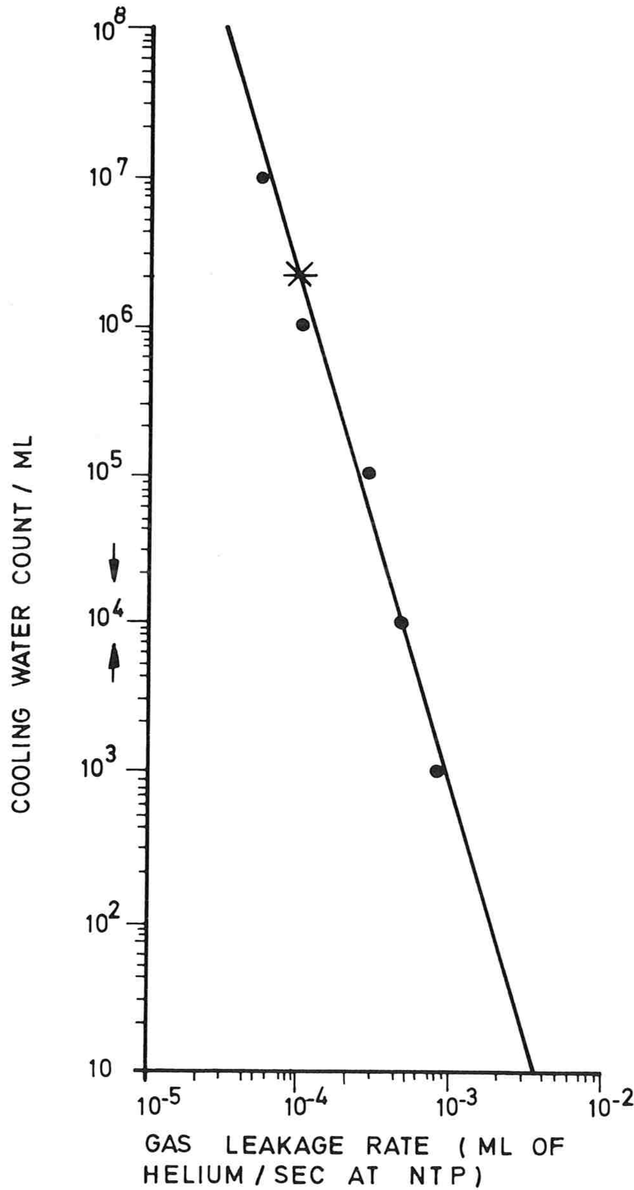


Figure 11. *Simultaneous determination of helium gas and bacterial can leakage. ⁎ maximum admissable helium gas leakage rate at NTP (normal temperature and pressure). → admissible number of bacteria in can cooling water (Perigo, personal communication).*

cooling process should be followed by a rapid drying of the containers, avoiding rough handling during post-cooling transport and storage (*5,26*) and (*f*) any part of the container handling equipment, including the postcooling unit, coming in contact with the seam or closure, should regularly be cleaned and disinfected (*20,21,22*) for bacterial spores may also survive, or may be adapted to survive a standard chlorination process.

ACKNOWLEDGMENT

We thank Mr. J. A. Perigo and coworkers of the Metal Box R&D Microbiological Department, Wantage, UK, for their permission for publication of the Fig. 5, 6, 7, 8 and 9 and some data concerning the manganese test for simultaneous determinations of water and microbial can leakage as given in Table 8. Besides, discussions of the mechanism of leaker spoilage of canned foods with the Metal Box collegues is gratefully appreciated. Data shown in Tables 4-7, 9-13 and

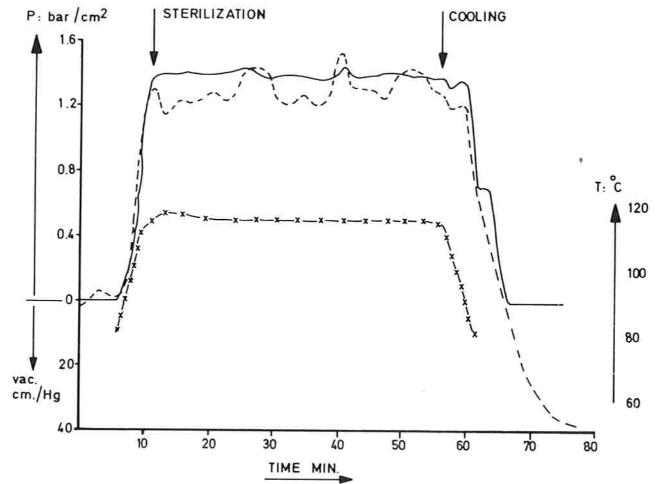


Figure 12. *T (temperature) P (pressure) diagram of the sterilization process of green peas packed in glass jars closed with omnia caps.* x —— x, *T autoclave;* ●——●, *P autoclave;* ---, *P head space glass jar.*

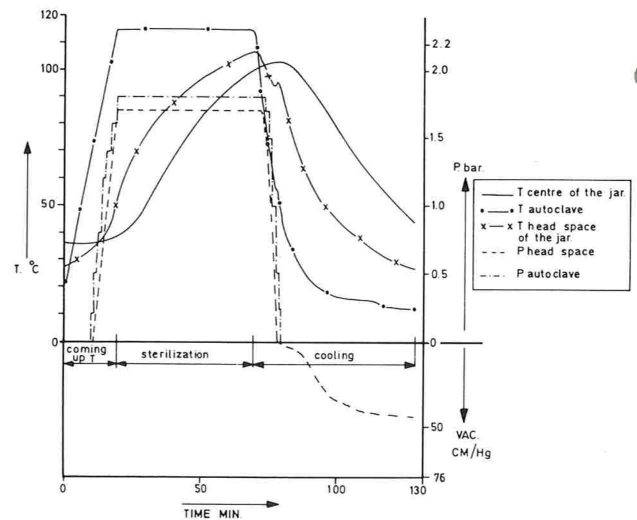


Figure 13. *T (temperature) P (pressure) diagram of the sterilization process of white beans and cut beans, packed in glass jars of 3/4 l and closed with pano caps.* —— *T center of the jar;* ●——● *T autoclave;* x —— x *T head space of the jar;* ---- *P head space of the jar;* —●—●—● *P autoclave.*

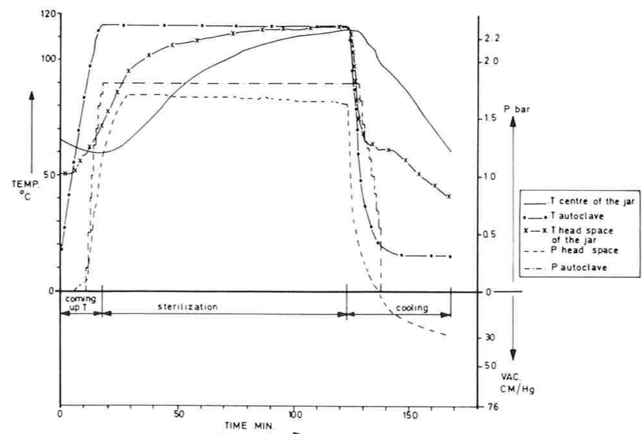


Figure 14. *T (temperature) P (pressure) diagram of the sterilization process of spinach, packed in glass jars of 3/4 l and closed with pano caps.* —— *T center of the jar;* ●——● *T autoclave;* x —— x *T head space of the jar;* ---- *P head space of the jar;* —●—●— *P autoclave.*

TABLE 15. *Ventilating glass-jar closure[a]; Leakage of A: water; B: air; C: bacteria.*

Enclosure	Ivac. cm Hg 20 C	Cooling		Post-cooling and incubation	
		A water (%)	B air (+ / −)	C bacteria (%)	B air (+ / −)
None	30	< 0.4	−	0.1	− / ∓
	60	2-20	−	< 0.4	− / ∓
Nylon, diam.	30	1	−	52	± / −
0.1 mm	60	50	−	8	∓ / ±
Cotton, diam.	30	0.4	−	16	− / ∓
0.4 mm	60	50	−	58	+

[a]Omnia, compound II.
A, Fluorescein; B, Loss of vacuum; C, 30 d 25 C.

TABLE 16. *Ventilating glass jar closure[a], air leakage.*

Enclosures	Vacuum cm Hg 20 C		Post-cooling C and incubation		
	A I	B cooling	min.	max.	ave.
None	30	32	25	35	30
	60	62	46	64	60
Nylon, diam.	30	32	20	40	33
0.1 mm	60	62	43	64	52
Cotton, diam.	30	32	26	40	34
0.4 mm	60	62	32	46	41

Spreading: A: ± 2 cm Hg, B: ± 3 cm Hg, C: 30 d, 25 C,
[a]Omnia compound III. I = Initial.

TABLE 17. *Ventilating glass jar closure[a], Biotest.*

Enclosures[b]	Bacterial reinfection[c] (%)
None	< 0.4
Vegetable filaments	30-35
Nylon, diam. 0.1 mm	40-50
0.15 mm	80
Cotton, diam. 0.4 mm	16-20
Silk, diam. 0.3 mm	85
0.4 mm	100

[a]Omnia, compound IV.
[b]I vac: 30-40 cm Hg 20 C, loss of vac: − / ±.
[c]Incubation 30 d, 25 C.

Fig. 2, 10 and 11 have been published previously. Presented in the symposium, "Prevention of Post-Processing Microbial Contamination of Thermally Processed Foods in Hermetically Sealed Containers," at the 39th Annual Meeting of the Institute of Food Technologists, St. Louis, MO, June 10-13, 1979.

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3-A Sanitary Standards for Cottage Cheese Vats

Number 38-00

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

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Cottage cheese vat specifications heretofore or hereafter developed which so differ in design, material, and construction or otherwise, so as not to conform to the following standards, but which are, in the opinion of the manufacturer or fabricator, equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.
Scope

A.1
These standards cover the sanitary aspects of open top-type vats used in the batch production of cottage cheese curd.

A.2
In order to conform with these 3-A Sanitary Standards, cottage cheese vats shall comply with the following design, material and fabrication criteria.

B.
Definitions

B.1
Product: Shall mean skim milk, cottage cheese curd and whey.

B.2
Cottage Cheese Vat: (Referred to hereinafter as "vats.") Shall mean a vessel in which cottage cheese is produced and may include (1) means for heating and/or cooling, (2) means for agitation and (3) means for curd handling within the vessel.

B.3
Surfaces

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

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

B.4
Lining: Shall mean all surfaces used to contain the product.

B.5
Shell: Shall mean the outer jacket.

B.6
Rail: Shall mean that portion of the metal used to join the lining to the shell.

B.7
Outlet: Shall mean an opening in the lining and the passage for the product to the exterior of the vat. The outlet passage starts at the opening in the lining and terminates at the connection for the outlet valve.

B.8
Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

B.9
Tools: Shall mean the blades or tines immersed in the product for cutting, stirring, forking or pushing.

C.
MATERIALS

C.1
Product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E.), or metal which under conditions of intended use is at least as corrosion-resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1
Rubber and rubber-like materials may be used for protective caps for sanitary tubes, fittings, vents, O-rings, seals, gaskets, and parts used in similar applications.

C.1.2
Rubber and rubber-like materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for rubber and rubber-like materials, Number 18-00.

C.1.3
Plastic materials may be used for protective edges on tools integral with the vat, moving component parts, agitator seals, agitator bearings, protective caps for sanitary tubes, fittings, vents, O-rings, seals or gaskets.

[1]*The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table2-1, pp. 18-19. Available from: American Iron and Steel Institute, 1000 16th Street NW, Washington, DC 20036.*

[2]*Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

C.1.4

Plastic materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for plastic materials, Number 20-11.

C.1.5

Except for the protective caps provided for in C.1.1 and C.1.3, sanitary fittings shall be made of materials provided for in the 3-A standard for sanitary fittings, Number 08-17.

C.1.6

Where materials having certain inherent functional properties are required for bearing surfaces and rotary seals, carbon materials may be used.

C.2

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

D.

FABRICATION

D.1

All product contact surfaces shall have a finish at least as smooth as a No. 4 ground finish on stainless steel sheets and be free of imperfections such as pits, folds and crevices in the final fabricated form. (See Appendix, Section F.).

D.2

Permanent joints in product contact surfaces shall be continuously welded except that rolled-on sanitary pipeline ferrules or flanges may be used on connections. Welded areas of product contact surfaces shall be at least as smooth as a surface finish equivalent to 150 grit or better as obtained with silicon carbide properly applied on stainless steel sheets.

D.3

Product contact surfaces of a vat not designed to be mechanically cleaned shall be easily accessible for cleaning and inspection either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.4

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

D.5

Product contact surfaces shall be self-draining except for normal clingage. This shall be accomplished by either having the bottom of the lining slope toward the outlet not less than 4 inches per 50 feet or by having a lifting device to raise one end of the vat to the required minimum slope.

D.5.1

An integral lifting device shall be made a part of each vat that does not have sufficient slope to be self-draining. Lifting devices shall mean any system that raises the vat for proper drainage and which does not at any time project over vat rail.

D.5.2

The construction of the vat shall be such that the lining will not sag, buckle, or become distorted in normal use to the extent drainage is affected.

D.6

Gaskets shall be removable. Gasket retaining grooves shall be no deeper than their width and shall not be less than 1/4 inch wide except for those standard O-rings smaller than 1/4 inch.

D.7

Internal angles of 135° or less on product contact surfaces of the lining shall have radii not less than 3/8 inch and all other internal angles of 135° or less on product contact surfaces shall have radii not less than 1/4 inch, except that:

D.7.1

The radii in gasket grooves or gasket retaining grooves, except those for standard 1/4 inch and smaller O-rings, shall not be less than 1/8 inch.

D.7.2

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

D.8

There shall be no threads on product contact surfaces.

D.9

The rail of the vat shall:

D.9.1

Be integral with or welded to the lining.

D.9.2

Be sloped so that drainage is away from the lining.

D.9.3

Unless the junction of the rail and the shell is welded, extend downward at least 1/2 inch.

D.9.4

Be continuously welded at the junction with the shell or effectively sealed with a non-toxic waterproof mastic material, which will set without cracking and present a smooth surface.

D.10

Sanitary fittings and connections shall conform with the applicable provisions of the 3-A standard for sanitary fittings, Number 08-17.

D.11

Vat Supports: The means of supporting a vat shall be one of the following:

D.11.1

With legs: Adjustable legs shall be of sufficient number and strength and so spaced that the filled vat will be adequately supported. Legs shall be smooth with rounded ends and have no exposed threads and shall be of sufficient length to provide (1) that the

distance between the lowest product contact surface of the lowest outlet and the floor will be at least 2 ½ inches and (2) a clearance of at least 6 inches between the floor and the lowest part of the shell with the exception of the portion of the shell at the outlet. Legs made of hollow stock shall be sealed.

D.11.2

With legs at one end and a lifting device at the other end: The distance between the floor and lowest part of the shell and/or any supporting member shall be at least 2 inches, provided the lifting device can raise that end of the vat a distance that is not less than 1/2 inch multiplied by the length of vat in feet.

D.11.3

Mounted permanently on a slab or island: The base of the vat shall be such that it may be sealed to the mounting surface. (See Appendix, Section G.).

D.12

Non-Product Contact Surfaces. Non-product contact surfaces shall comply with the following:

D.12.1

They shall be smooth, be readily cleanable and have no places where liquids and/or solids may accumulate.

D.12.2

Surfaces to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[3] specifications A296-68 and A351-70.

F.

Surface finish equivalent to 150 grit or better, as obtained with silicon carbide properly applied on stainless steel sheets, is considered in compliance with the requirements of Section D.1 herein.

G.

SLABS OR ISLANDS

When a vat is designed to be installed on a slab or an island, the dimensions of the slab or island should be such that the vat will extend beyond the slab or island at least 1 inch in all horizontal directions.The slab or island should be of sufficient height so that the lowest product contact surface of the lowest outlet connection is not less than 2 1/2 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the outer shell of the vat and the slab or island should be sealed.

These standards shall become effective August 1, 1980.

[3]*Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.*

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Milwaukee Health Department
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Milwaukee, Wisconsin 53202

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Calendar

June 15-18--ASSOCIATION OF FOOD AND DRUG OFFICIALS, 84th Annual Conference. Kirkwood Motor Inn, Bismarck, ND. Contact: Association of Food and Drug Officials, PO Box 3, Barrington, RI 02806.

June 15-18--75th ANNUAL MEETING, AMERICAN DAIRY SCIENCE ASSOCIATION. Virginia Polytechnic Institute and State University, Blacksburg, VA. Further details will be available once the program is finalized.

June 15-18--AMERICAN SOCIETY FOR AGRICULTURAL ENGINEERS, Summer Meeting. Convention Center, San Antonio, TX. Theme, "Managing Resources in Transition: Agriculture's Challenge for the 80's." Contact: Roger R. Castenson, ASAE, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300.

June 19-20--CLEAN AIR ACT SEMINAR. Washington, DC. Sponsored by Government Institutes, Inc. Fee: $395. Contact: Government Institutes, Inc., PO Box 5918, Washington, DC 20014.

June 24-25, 26-27--SEPARATION WORKSHOPS. Rockville, MD. Sponsored by LKB Instruments, Inc. Fee: $150. Contact: Workshop Director, LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, MD 20852, 301-881-2510.

June 29-July 3--WORLD CONGRESS ON FOODBORNE INFECTIONS AND INTOXICATIONS, Berlin, West Germany. Sponsored by the Institute of Veterinary Medicine, Robert Von Ostertag-Institute of the FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses. Contact: Dr. K. Gerigk, Secretary General, World Congress on Foodborne Infections and Intoxications, D-1000 Berlin 33, Thielallee 88-92, Postfach 33 00 13.

July 6-11-XI INTERNATIONAL SYMPOSIUM ON PSYCHROTROPHIC MICROORGANISMS IN SPOILAGE AND PATHOGENCITY. Aalborg Universitetscenter, Sohngaardsholmsvej 57, Aalborg, Denmark. Sponsored by International Association of Microbiological Societies, Committee on Food Microbiology and Hygiene. Contact: Secretariat, IAMS, XI International Symposium, Institute of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870, Copenhagen V, Denmark.

July 7-11--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. Stark Technical College, Canton, OH. Sponsored by the National Sanitation Foundation. For more information, see Aug. 11-15 entry.

July 7-25--DAIRY LABORATORY WORKSHOP, University Park campus, The Pennsylvania State University, State College, PA. Workshop will consist of two weeks of lectures and laboratories, followed by a third week of reviewing and completing regulatory tests, examinations. Fee for first two weeks, $250. For those wishing to take certification examination in third week, fee is an additional $100. Contact: Agricultural Conference Coordinator, 409 J. O. Keller Building, University Park, PA 16802, 814-865-9547.

July 14-15, 16-17--SEPARATION WORKSHOPS. Research Triangle, NC. Sponsored by LKB Instruments, Inc. For more information, see November 18-19 entry.

July 26-31--IAMFES ANNUAL MEETING, Red Carpet Hotel, Milwaukee, WI. Contact: Earl Wright or Jan Richards, IAMFES, PO Box 701, Ames, IA 50010, 515-232-6699.

July 28-29, 30-31--SEPARATION WORKSHOPS. St. Louis, MO. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

July 28-Aug. 1--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. Harvard University, Cambridge, MA. Sponsored by the National Sanitation Foundation. For more information, see Aug. 11-15 entry.

Aug. 4-8--ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Summer course, Massachusetts Institute of Technology, Cambridge, MA 02139. Contact: Director of Summer Session, RM E19-356, MIT, Cambridge, MA 02139.

Aug. 4-8--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. Highline Community College, Midway, WA. Sponsored by the National Sanitation Foundation. For more information, see Aug. 11-15 entry.

Aug. 11-15--SANITATION ASPECTS OF FOOD FACILITY PLANT PREPARATION & REVIEW. State Technical Institute, Memphis, TN. Sponsored by the National Sanitation Foundation. Contact: NSF Education Service, National Sanitation Foundation, PO Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Aug. 18-19, 20-21--SEPARATION WORKSHOPS. Houston, TX. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Sept. 8-9, 10-11, SEPARATION WORKSHOPS. Los Angeles, CA. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Sept. 15-16, 17-18--SEPARATION WORKSHOPS. San Francisco, CA. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Sept. 15-19--CANADIAN INSTITUTE OF PUBLIC HEALTH INSPECTORS, 41st Annual Conference. Holiday Inn, Kingston, Ontario. Contact: William D. Vaughan, RR 1, Napanee, Ontario, Canada K7R 3K6.

Sept. 26--FOOD DEHYDRATION SYMPOSIUM, part of Kansas State University's Focus on Food Science Symposium series. Contact: Dr. D. Y. C. Fung, 913-532-5654, or Dr. D. S. Chung, 913-532-5580, Co-chairmen, Call Hall, KSU, Manhattan, KS 66506.

Sept. 29-30--CALIFORNIA ASSOCIATION OF DAIRY, MILK SANITARIANS, ANNUAL MEETING. Sacramento Inn, Sacramento, CA. Contact: John C. Bruhn, Extension Food Technologist, 101 Cruess Hall, Univ. of California, Davis, CA 95616, 916-752-2192.

Sept. 29-Oct. 1--ASAE NATIONAL ENERGY SYMPOSIUM. Co-sponsored by USDA, DOE, and 19 other organizations. Radisson Muehlebach Hotel, Kansas City, MO. Contact: Mark A. Purschwitz, American Society of Agricultural Engineers, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300.

Oct. 6-7, 8-9--SEPARATION WORKSHOPS. Chicago, IL. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Oct. 6-8--ENERGY AND THE FOOD INDUSTRY. International Symposium sponsored by Commission Internationale des Industries Agricoles et Alimentaires (CIIA), Madrid, Spain. Contact: CIIA, B.P. 470-08, 75366 Paris Cedex 08.

Oct. 8-9--NEBRASKA DAIRY INDUSTRIES ASSOCIATION, 26th ANNUAL CONVENTION. Regency West, I-680 and Pacific Street, Omaha, NE. Contact: T. A. Evans, Exec. Secretary, 116 Filley Hall, East Campus, University of Nebraska-Lincoln, Lincoln, NE 68583.

Food and Fieldmen

The new IAMFES magazine, *Food and Fieldmen,* will address many of the same concerns as does the current IAMFES publication, *Journal of Food Protection. Food and Fieldmen,* however, will provide articles of immediate interest and application to the work of the practicing sanitarian, fieldman, and quality control person.

As such, it will complement the scientific *Journal of Food Protection,* which will continue to offer the latest research in milk and food sanitation and technology.

In addition to articles, *Food and Fieldmen* will contain departments now included in the *Journal,* but they'll be expanded in the new magazine to offer readers more complete information about news, events, and others in the field. Among the expanded departments will be news about IAMFES affiliate members, meetings, and events; Association events; new product news; excerpts from such publications as the Center for Disease Control's "Morbidity and Mortality Weekly Report," and the Federal Register. New 3A and E-3A Sanitary Standards and amendments to existing standards will also be included in *Food and Fieldmen.*

A sample issue of *Food and Fieldmen* will be sent out this month, and regular publication will begin with the January, 1981 issue. Return the portion below for a copy, or to request additional information about IAMFES the *Journal of Food Protection,* and the new *Food and Fieldmen.*

Please send more information about *Food and Fieldmen*...

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AABP Plans July Seminar

"Microbiology of Milk — Wet Lab," a seminar planned by Region 5 of the American Association of Bovine Practitioners, AABP, will follow the summer meeting of the National Mastitis Council and the IAMFES Annual Meeting.

The seminar is planned for August 1 at the Red Carpet Hotel in Milwaukee, WI. Sessions will run from 8 a.m. to 12 p.m. Time will also allow discussion with resource persons following the sessions.

Among topics to be discussed will be equipment needs for a practical clinical laboratory, sample collection and transport, bulk milk culturing, special media, and sensitivity testing. Laboratory procedures will also be discussed.

Registration is limited to 35 persons and the cost is $20 per person. For more information, contact: Darrel E. Johnson, DVM, 109 East Wisconsin St., Weyauwega, WI 54983.

IDF to Meet in England

The 64th Annual Session of the International Dairy Federation (IDF) is scheduled for Bristol, England, September 7-12, 1980.

Persons interested in the meeting or the development of the U.S. National Committee of IDF in the U.S.A. (INCUS) should contact Harold Wainess, INCUS Secretary, 464 Central Ave., Room 24, Northfield, IL 60093, 312-446-2402.

Wainess notes that establishment of a permanent US National Committee on the IDF is still in the formative stages and INCUS encourages as many US participants at the meeting as possible.

Food and Fieldmen Ready This Month

Food and Fieldmen makes its debut this month, in a special sample issue.

Milk, food and environmental protection, on a practical level, is the focus of the new magazine, published by IAMFES. Articles of immediate interest and application to the work of the practicing sanitarian, fieldman, and quality control person will be featured in each month's issue.

Regular publication of *Food and Fieldmen* begins with the January, 1981 issue. For additional information on the new magazine, see p. 503 of this issue.

Sanitarian Registration Council to Meet in Milwaukee

The Council of State Sanitarian Registration Agencies will meet during the IAMFES Meeting in Milwaukee, WI.

The Council will meet at 1:15 p.m. July 26 and 27, at the Marc Plaza Hotel.

For additional information, contact: Henry D. Wilson, chairman, Council of State Sanitarian Registration Agencies, 400 E. Gray Street, Louisville, KY 40222.

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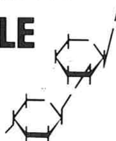

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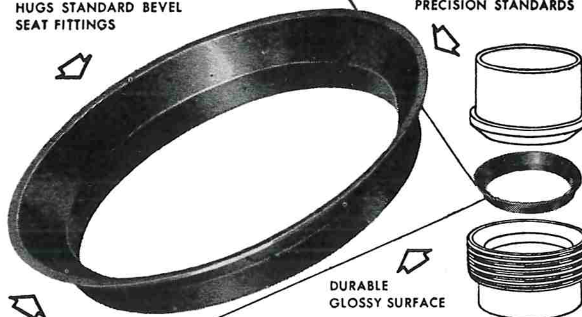
HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



LOW COST . . . RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these **SNAP-TITE** *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES SELF-CENTERING SNAP-TITE® Gaskets

SIZES 1" - 1½"
2" - 2½" - 3" - 4"

*MADE FROM **TEFLON®**

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

• *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

* Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

A HEAVY DUTY SANITARY LUBRICANT

Available in both
SPRAY AND TUBE

All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

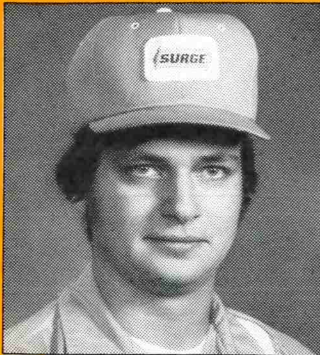
For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113

SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY



Richard Athey, Dairy Equipment Serviceman

Richard Athey grew up at his father's dairy equipment dealership in Berlin, Pennsylvania. He has attended the Surge Training Center near St. Charles, Illinois, on three separate occasions, receiving performance certificates in both Basic and Advanced Refrigeration and in Advanced Dairy System Trouble Shooting. While in high school, Mr. Athey worked weekends and summers as a dairy equipment serviceman and has continued this profession on a full-time basis for the past six years.

"Dairymen have a lot on their hands these days just trying to manage their operations for better performance. The last thing they want to worry about is the efficiency of their milking equipment. They have a right to expect this important system to operate at top efficiency, and that's where a serviceman can make a big difference."

Preventing Trouble Before it Starts

"Along with emergency service and repairs, my activities include checking the entire milking system periodically to find small problems before they become major breakdowns. The regularity of these preventative maintenance inspections is determined by the dairyman and vary from one dairy to the next. However, one way or another, certain things must receive regular, periodic attention to assure the system is working properly.

"These include:

- Vacuum System — Checking vacuum pump oil level, pump oilers, regulator and filters is essential to consistent vacuum operation.
- Milking System — Since this system can have a direct affect on udder health, it is most important to examine inflations, pulsator ratios, pulsator timing and milking vacuum levels.
- Sanitation Equipment — There is a direct relationship between milk quality and cleanliness so making checks of pipe line washers, hot water heaters, as well as analyzing the water help protect the milk from contamination.
- Refrigeration — Keeping tabs on the cooling time and blend temperatures can help prevent a major loss of milk through cooling breakdowns.

"No matter if these things are all checked by the serviceman, or some of them are accomplished by the dairyman, they are necessary to keep the system working right and prevent major problems from developing. For a car or tractor to work right, it must be kept in proper tune with all lubricants provided in sufficient quantity. The milking system is even more important than these machines, and that means it must be tuned properly to perform right.

"I firmly believe that if we compared a group of dairies with regular maintenance programs against a group without it, we would find that most of our major equipment outages and emergency calls would go to the last group. The investment in regular, preventative maintenance is money well spent because it protects the dairyman and his herd from unexpected and often costly emergencies.

"Why is this so? Because the milking system is the only equipment on the farm that works on live tissue, namely the cow's udder and teats. If the pulsation goes bad or improper vacuum level or fluctuating vacuum occurs, the herd could develop very costly udder problems. This just doesn't need to happen if the equipment is checked and adjusted regularly. After checking the system with our testing equipment such as the Levograph, we can correct it right away before it affects the herd."

Why We Care

"I care about the job I do for the dairyman. He has a big investment and you have to help him protect that investment. It's needless to say we are like partners. If his operation suffers, it won't be long before I'm suffering along with him. If he's out of work, so am I, and that means one thing to me: I'll do everything in my power to help the dairyman succeed. Working together, we do the best we can to improve what we do, and that helps the dairy industry succeed in the process."