Journal of
Milk and Food
Technology

Official   INTERNATIONAL ASSOCIATION MILK, FOOD & ENVIRONMENTAL SANITARIANS   **Publication**

METHODS FOR PRODUCTION OF HIGH QUALITY RAW MILK

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

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PUBLISHED BY

INTERNATIONAL ASSOCIATION OF MILK, FOOD, AND ENVIRONMENTAL SANITARIANS, INC.

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International Association of Milk, Food, and Environmental Sanitarians, Inc.
Box 437, Shelbyville, Indiana 46176

Prices: Single Copies $2.00 each—25-100 Copies $1.75 each,
100 or More Copies $1.50 each

Journal of
MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION

Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

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WHAT SEAFOOD PROCESSORS SHOULD KNOW ABOUT VIBRIO PARAHAEMOLYTICUS[1]

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ABSTRACT

Information on *Vibrio parahaemolyticus* that is pertinent for its control in food processing operations is compiled and discussed in this paper. The growth potential of this organism and requirement for NaCl are discussed in some detail. Effects of temperature, pH, and antimicrobial agents are also presented.

Repeated outbreaks of gastroenteritis, caused by *Vibrio parahaemolyticus* in the United States *(24, 25, 26)* point to the need for control measures. Despite the numerous publications on *V. parahaemolyticus* scattered throughout the literature, information pertinent to the control of this organism is scarce. The intent of this publication is to bring together information that will help guide the seafood processors in meeting this new challenge.

Readers interested in additional information are referred to two recent r e v i e w s by Fishbein and Olson *(9)*, and Nickerson and Vanderzant *(27)*. Selected publications on the distribution and incidence in seafoods *(3, 4, 8, 14, 17, 31, 33, 35)* and the isolation and identification methods *(2, 23, 29, 30, 32, 33)* are listed in the references.

GROWTH

The amazingly rapid growth rate of *V. parahaemolyticus* is perhaps one of the most important characteristics to be considered. Aiso *(1)* grew *V. parahaemolyticus* strain No. 7 in brain-heart infusion broth (pH 7.9), plus 1.5% NaCl, on a shaker at 37 C. Growth was measured spectrophotometrically at 470 nm and by the plate count. The generation time thus obtained at the logarithmic growth phase was 7.6 min. This was claimed to be the shortest ever recorded for any bacteria. He also noted that growth of *V. parahaemolyticus* was equally rapid in seafoods, and the generation time of this organism in inoculated squid was 13 min.

Although we could not duplicate the 7.6-min generation time under identical conditions with 4 different strains of *V. parahaemolyticus*, the average generation time obtained was still a remarkably short 13.6 min *(unpublished data)*.

The rapid growth of this organism might also have been responsible for the incriminated seafoods' often fresh and unspoiled appearances *(1)*. Aiso *(1)* showed that at 37 C, *V. parahaemolyticus* strain No. 7 increased in number from 10^2 to 10^4 within 1.5 hr in the inoculated squid; and it increased to 10^7 after 4.5 hr. In the same time period, the count of indigenous microorganisms barely increased from 10^2 to 10^3; and the total volatile bases, or the chemical indices of spoilage, had hardly increased.

After the Louisiana outbreak of *V. parahaemolyticus* gastroenteritis *(25)*, no mention of the incriminated shrimp having an off odor or flavor was made by those interviewed. A number of them, however, noted that the shrimp they ate appeared undercooked (Center for Disease Control, Atlanta, *personal communication*). Our unpublished data on volatile degradation products of *V. parahaemolyticus* in sterile fish showed that the compounds identified were similar to those reported for naturally spoiling fish *(22)*.

The lack of putrefactive or spoiled appearance of incriminated seafoods, therefore, appears to be due to quantitative rather than qualitative difference, i.e., the *V. parahaemolyticus* population could have reached dangerous proportions before signs of their presence might be detected.

SODIUM CHLORIDE REQUIREMENT

V. parahaemolyticus strains r e q u i r e NaCl for growth and maintenance of viability *(10, 18)*. The halophilism of this organism was one of the early characteristics recognized by the Japanese investigators, and it helped to establish this organism as a new agent of food poisoning *(10)*.

The requirement for NaCl perhaps reflects the marine origin of this organism. As with the other marine bacteria, *V. parahaemolyticus* requires NaCl partially for osmotic balance, and lysis in hypotonic solutions *(5, 13)*. Hidaka and Kakimoto *(12)* compared the osmotic fragilities of a non-marine bacterium, *P. fluorescens*, a marine bacterium, *Pseudomonas* 1055-1, and *V. parahaemolyticus*. *V. parahaemolyticus* lysed in NaCl or KCl concentration of 100 mM or less but required 1 mM or less of divalent cations, $MgCl_2$ or $CaCl_2$, before lysis could be induced.. *P.*

fluorescens was not sensitive to hypotonic conditions. The marine *Pseudomonas* 1055-1, however, was more sensitive than *V. parahaemolyticus;* and lysis could be induced by 600 m$_M$ or less of NaCl or KCl and 10 m$_M$ or less of MgCl$_2$ or CaCl$_2$.

The concentration of NaCl tolerated by suspected isolates serves as an important differential criterion (2, 30). The maximum NaCl concentration tolerated by *V. parahaemolyticus* is 8%, while the closely related organism, *V. alginolyticus* can grow in 10% NaCl. The optimum concentration of NaCl for *V. parahaemolyticus*, however, is between 2 and 4%. In fact, *V. parahaemolyticus* was reported to grow poorly in foods containing 5% or more of NaCl (16).

REACTION OF THE SUBSTRATE

V. parahaemolyticus prefers an alkaline pH. The recommended pH for the culture media is 7.4 to 8.6 (2).

Kodama (16) claimed a correlation between the ability of a food to support growth of *V. parahaemolyticus* and its pH. Among 26 seafoods; 27 vegetables and pickles; and 9 meat, poultry, and dairy products common to the Japanese diet, he demonstrated that no food with a pH below 5.8 supported growth of *V. parahaemolyticus*. Maximum growth was obtained in uncooked octopus and marinated egg, and their respective pH values were 7.7 and 8.5.

The pH, however, may be one of many factors that influence *V. parahaemolyticus*. Some foods that did not support maximum growth of *V. parahaemolyticus* still had pH values in the range of 6.0 to 7.9. Cooked beans, despite their pH of 7.9, failed to support growth of *V. parahaemolyticus*.

Susceptible foods according to Kodama, are "proteinatious," with NaCl contents of 1 to 3%, and pH above 5.8. A food with a pH value below 5.0 and NaCl content above 5% is not l i k e l y to support growth of *V. parahaemolyticus*. He noted, however, that some foods might not attain the inhibitory concentrations of salt and pH, shown by the finished product, during processing. Therefore, one must also take the history of each food into account.

The effect of pH on *V. parahaemolyticus* strain O in shrimp homogenate was studied by Vanderzant and Nickerson (34). They showed that viability was not affected by pH between 6 and 10 during a 2-hr test period. However, rapid inactivation took place at pH 5.0.

GROWTH TEMPERATURES

The reported optimum temperature for growth varies from 35 to 37 C (19). Given the rapid growth rate of this organism, the difference tends to be insignificant between optimum and sub-optimum temperatures. The minimum and maximum growth temperatures reported are 5-8 and 42-45 C, respectively (19). Although the strains we examined did not grow at temperatures below 10 C, and the generation time was twice as long at 20 C than at 37 C (unpublished data), the minimum growth temperature could be lowered in fish substrate, as shown by some *Salmonella* strains (20).

HIGH TEMPERATURES

V. parahaemolyticus is very heat sensitive and can be inactivated by mild heat. At 48 C, 3 C above its maximum growth temperature, approximately 90% of the cells were inactivated in <1 hr in a broth, and in <1.5 hr in fish homogenate (7). After heating at 60 or 80 C for 15 min, no survivors could be detected in a shrimp homogenate inoculated with 500 cells/ml. Only when the cell concentration was increased to 2×10^6/ml were there detectable survivors after 15 min at 80 C; but no survivors were detected after 1 min at 100 C (33).

The sensitivity of this organism to heat probably has prevented untold numbers of *V. parahaemolyticus* outbreaks in this country. In Japan, where two-thirds of all gastroenteritis during the summer months has been attributed to *V. parahaemolyticus*, the large consumption of seafoods, coupled with the custom of consuming raw seafoods, has been thought as the probable cause.

All reported outbreaks in the United States, however, were due to cooked crustaceans, except a suspected case involving oysters and raw crab used in "poi." It is noteworthy that such seafoods are normally consumed without further cooking.

Another factor that must be considered is that *V. parahaemolyticus* gastroenteritis may not be exclusive to seafoods. Again in Japan, salted cucumbers have been incriminated in a *V. parahaemolyticus* outbreak (10). High protein foods of alkaline pH, such as raw egg and egg products (pH 7.9 to 8.5) had been shown to support the growth of *V. parahaemolyticus* (16).

LOW TEMPERATURES

The cold sensitivity of this organism was recognized early by the reduction of *V. parahaemolyticus* gastroenteritis during winter months (28). The extent of inactivation due to low temperature, however, is far less than that due to heat. Two outbreaks reported in the United States in 1972 involved frozen shrimp, and viable *V. parahaemolyticus* cells were recovered from the frozen samples (26). Conflicting

evidence exists, but in general, the low temperature inactivation of *V. parahaemolyticus* is a negative function of the temperature. Matches et al. *(21)* subjected 13 *V. parahaemolyticus* strains in fish homogenate to 0.6, −18, and −34 C and noted that at 0.6 C, a 2.0 to 6.4 log reduction was obtained in 26 to 48 days. The log reduction values of 2.2 to 6.2 at −18 C were attained in 12 to 19 days, and the same reduction values at −34 C were reached before the 12th day. Vanderzant and Nickerson *(34)* subjected their Gulf Coast isolate (*V. parahaemolyticus strain O*) to 3, 7, 10, and −18 C in whole and homogenized shrimp. In whole shrimp, the initial loss of viability was rapid and resulted in a 2-log reduction within 2 days. After this initial loss, no further reduction was noted during 6 additional days of observation. The loss of viability in shrimp homogenate was not as great as in the whole shrimp, and no more than a 2-log reduction was observed in 8 days. It is also interesting to note that the strain Vanderzant and Nickerson studied was more readily inactivated at 3 than at −18 C. The data, therefore, suggest that refrigeration temperatures may be more detrimental to *V. parahaemolyticus* than freezing. A similar study by Covert and Woodburn *(7)*, however, showed that *V. parahaemolyticus* strain SB04-422 in trypticase soy broth with 6% NaCl was inactivated more readily at −18 than at −5 C, and −5 was more detrimental to this organism than 5 C.

DISINFECTION

Effectiveness of various antibiotics, detergents, disinfectants, and food preservatives against *V. parahaemolyticus* has been thoroughly investigated in Japan *(36)*. Information that may be applicable to food handlers is summarized below.

Among 12 antibiotics tested, chlorotetracycline was the most effective and penicillin the least. The minimum inhibitory concentrations (MIC) were 0.5 µg/ml and 75 µg/ml respectively. The MIC for most other antibiotics were <10 µg/ml.

Among 14 food preservatives, the most effective one was propyl-p-hydroxybenzoate with the MIC of 0.05 to 0.1 mg/ml, and the least effective was potassium sorbate with the MIC of 2.5 to 10.0 mg/ml. Glycerine was shown to be injurious to *V. parahaemolyticus* at 30% concentration, but not at 15% *(6)*.

Sodium hypochlorite (12% available chlorine), diluted in 3% NaCl to 1/3,000th of its original strength, inactivated *V. parahaemolyticus* within 5 min. In 15% methyl or ethyl alcohol, *V. parahaemolyticus* was inactivated within 30 min. Hydrogen peroxide was also lethal to *V. parahaemolyticus;* a 0.5% solution inactivated the organism within 2.5 min.

Some heavy metals such as gold, silver, and copper were found to be bacteriostatic to *V. parahaemolyticus;* but alminium, tungsten, and tin had no effect.

V. PARAHAEMOLYTICUS CONTROL MEASURES

The major natural reservoir of *V. parahaemolyticus* appears to be the sea and the marine animals we harvest. Preventing contamination of raw materials, therefore, would be nearly impossible. To safeguard seafoods, efforts should be directed to preventing contamination of the finished product, especially those foods that are to be consumed without further cooking. Seafood processors must eliminate the time-temperature abuse. This is essential in controlling the organism which can multiply so rapidly.

Although the organism may originate from the sea, it may find the processing plant environment favorable and may establish a secondary source of contamination somewhere in the plant. How such an ecological niche for *V. parahaemolyticus* may be established was shown by Kaneko and Colwell *(15)*. They investigated the seasonal fluctuation of *Vibrio* species, including *V. parahaemolyticus*, in Chesapeake Bay and found the sediment serving as the reservoir. The organisms were released from the sediment, attached themselves to the plankton and multiplied along with the blooming plankton population during the summer, then resettled to the sediment with the dying plankton in winter.

V. parahaemolyticus was not shown to permanently establish itself in human gut *(28)*. Nevertheless, 0.68 to 3.3% of seafood handlers examined in Japan during summer months harbored *V. parahaemolyticus*, in contrast to 0.33% for the control *(11)*.

V. parahaemolyticus is sensitive to heat, disinfectant, low temperatures, low pH, and tap water. However, none of these treatments, except heat, would inactivate *V. parahaemolyticus* to a safe level. To prevent further outbreaks involving seafoods consumed without further cooking, a terminal heating, followed by adequate refrigeration or freezing, is advisable.

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EFFECTS OF COOKING AND RINSING ON THE PROTEIN LOSSES FROM BLUE CRABS[1, 2]

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ABSTRACT

The effects of cooking blue crabs at two temperatures (100 C and 121 C) on the amount of cook loss and concurrent protein loss were studied under controlled pilot plant conditions. The 121 C cook temperature resulted in a greater volume of cook loss fluids which contained a greater amount of protein. Centrifugation of the cook loss and analysis of the supernatant fractions showed that the protein in the supernatant from the 121 C cook was significantly greater than in the supernatant from the 100 C cook. Percent protein in the solids component of the cook loss showed an inverse relationship being slightly, but not significantly, higher in the solids from 100 C cook than in the 121 C cook. Under pilot test conditions, rinsing samples of fresh picked crab meat with tap water resulted in protein losses of 15.2% for the 100 C cook and 12.6% for the 121 C cook. Dipping crab meat samples in a salt brine solution resulted in protein losses of 11.2% for the 100 C cook and 7.3% for the 121 C cook. Higher protein losses during the early winter season were attributed to seasonal variation in the physiological condition of the blue crab.

Little research has been done characterizing the wastes from blue crab processing. The liquid which is produced during processing of blue crabs (*Callinectes sapidus*) is not generally utilized but is discharged from the processing plant as waste material. Approximately 86% of the whole crab is not used for human consumption. Solid wastes have been processed and sold as animal feed or fertilizer. The liquid losses from blue crab processing can be generated during washing, rinsing, cooking, meat extraction, and, sometimes, cooling processes. Major concerns, therefore, are to find ways of either reducing or eliminating production of waste materials, *per se*, or to find uses for these wastes as saleable by-products. The liquid wastes have not been characterized as to their proximate composition.

Processes to which fish and shellfish are subjected, such as cooking, chilling, and freezing, have an effect on the amount of protein that will be lost. Major losses can be attributed to contact with processing waters or by direct loss of moisture from the muscle due to the type of process. Liquids lost during processing are considered to be exudates as contrasted to drip loss which is usually associated with the freeze-thaw cycle (8). Studies on the composition of drip loss showed that a relatively high amount of nitrogen-containing compounds were lost as the result of freezing and thawing crab meat (3). This research (3) further indicated that the amount of protein which was soluble in a 5% NaCl solution was 42.7 g/100 g for *Neptunus pelagicus*. These results generally confirmed those reported for fish (10) wherein 40, 53, and 48 g of protein/100 g of total protein were extracted with 5% NaCl from pomphet, surmai, and mackeral, respectively. Cod muscle resulted in 18.75 g protein/100 g being extracted, of which 22% was water soluble (sarcoplasmic) by using a 0.5 μ buffer solution (5). The extraction of soluble protein (7) from calico scallop meats resulted in from 24.4% to 31.5% of the total protein extracted, depending on the manner in which the raw product was processed (13). The level of extractable protein from various fishery products has been summarized (2), but data on the component proteins of the blue crab are virtually nonexistent. Commercial processing of the blue crab is believed to be prone to the loss of significant quantities of protein.

The commercial processing of blue crabs usually entails cooking live crabs under pressure (15 psi) for approximately 10 min at 121 C. In some states this time/temperature relationship is required by regulation. The crabs are subsequently air chilled but in some instances they are chilled by cold water rinsing. The meat is extracted (picked) from the chilled crab by either hand or mechanical methods. In recent years, the product is often exposed to tap water or salt brine during the extraction process. Briefly, the crab claws, legs, viscera, and back are removed and the component parts conveyed by flowing tap water. Subsequently, the meat is extracted by either hand picking or mechanical systems. Us-

ually the cores are hand picked while the meat from the claws is mechanically extracted by maceration and salt brine flotation to separate the meat and shell. More recently extensive mechanical separation has been accomplished on the whole crab with the extensive use of rinse waters and/or salt brine solutions. These systems expose the crab meat to liquids which potentially allow for extraction of soluble proteins.

Studies by the National Marine Fisheries Service (6) have indicated that the meat extracted from crabs cooked at 100 C for approximately 8 min was of a higher quality when subjected to frozen storage. However the need for further studies on this process were indicated. Determination of the adequacy of cook has been based on developing a firmness of texture and subsequent release of meat from the shell to allow easy picking and increased meat yields. Preliminary work in this laboratory has indicated that relatively low temperature/short time (100 C for 8 min) would not be sufficient to meet these criteria.

The objectives of this study were to investigate the amount of protein contained in the exudate from the cooking process and the effect of controlled rinsing and dipping operations on the amount of protein lost under simulated pilot processing methods for the blue crab.

EXPERIMENTAL PROCEDURES

Sampling

Since most crab processing plants are not equipped for precisely controlling the factors studied in this experiment, the work was done in the pilot laboratory of the Food Science Department, where exact temperatures and times, as well as volumes of water used or lost, could be recorded. Samples of live blue crabs were harvested from the sounds and rivers of Pamlico County, North Carolina. Crabs were transported to the laboratory in an open ice chest, underlined with ice and burlap within 12 hr after harvesting. When ambient temperatures exceeded 55 F (12.8 C), crabs were covered with wet burlap.

Sample preparation

Ten trials were completed over a period of 5 months. Market size crabs were randomly divided into two lots of 15 each, with no special selection being made for sex. Crabs were steamed from the live state within 24 hr following harvesting. Lots were randomly assigned in two treatments: (a) steaming at 100 C for 8 min and (b) steaming under pressure (15 psig) at 121 C for 10 min. After steaming, crabs were individually removed from the cooking container, drained free of excess fluid, and subsequently transferred to another pan and covered for air cooling overnight at 1.6 C. The liquid, along with any solidified material remaining in the cook loss fluids, was measured to determine volume of cook loss and refrigerated for subsequent analyses. Cook loss fluid from the steaming operation was blended in a Sunbeam blender, an aliquot taken for subsequent analyses and the remainder centrifuged for 10 min at 7970 $\times$ g at a temperature of 5 C. The supernatant was decanted and each fraction saved for subsequent analyses.

Macro- and micro-Kjeldahl analyses were done on the solid and supernatant fractions, respectively.

Chilled crabs were hand picked to remove only the lump meat. A 150-g sample of the meat was randomly selected from each lot (15 crabs) for use in the rinsing studies. Rinsing procedures were designed to simulate the rinsing of crab meat with tap water (as is practiced in some commercial operations).

To obtain values for protein lost during rinsing of the crab meat with tap water, a rinsing apparatus was constructed as illustrated in Fig. 1. A pressure pump was used to pressurize the 5-gal Nalgene bottle so that the water pressure could be controlled through a preset nozzle. The bottle was connected to the nozzle with 0.5-inch plastic tubing. A brass, garden-type spray nozzle was used to produce a spray to cover the desired circular area. The nozzle was adjusted to a height of 15 inches above the product and this produced a spray covering 8 inches in diameter. The pressure was set at 18 psig, which delivered 1550 ml water in 1.5 min. The crab meat sample was uniformly spread on a wire screen (8 inches in diameter, .0937 in² opening, Tyler equivalent 8 mesh) to allow good drainage and still restrict actual loss of whole pieces of meat. Each sample was rinsed for 15 min. The rinse water was collected and analyzed for nitrogen (protein).

A 150-g sample of the meat was randomly selected from each lot to study the effects of immersion of crab meat in NaCl brine (as encountered in the mechanical method (4) of meat extraction). To estimate losses of protein by the mechanical method of extracting crab meat, a simulated pilot system was used whereby 150 g crab meat were dipped into 1550 ml of an 8% NaCl brine solution for 10 sec. The meat was removed and allowed to drain until dripping essentially ceased. The brine solution was analyzed for total nitrogen.

Analytical methods

The solids from the centrifuged cook loss fluid were analyzed for nitrogen by the macro-Kjeldahl procedure with slight modification (1). The modifications consisted of drying the samples for 16 hr at 107 C in a vacuum oven and subsequently digesting in 40 ml of concentrated H_2SO_4, in the presence of a pre-measured catalyst (Kel-Pak, No 5). Rinse water, brine dip solution, and cook loss fluid (total and supernatant) were analyzed by the micro-Kjeldahl procedure (1). All samples were analyzed in duplicate and a blank was done with each determination. The pH values were determined with a Leeds and Northrup 7405 glass electrode pH meter. Moisture, fat, and ash content of fresh crab meat were determined (1) and statistical analyses were done on the data (10).

RESULTS AND DISCUSSION

The mean and standard error of the mean of the cook loss resulting from the cooking of whole crabs at 100 C and 121 C are given in Table 1. Although the amount of fluid which was collected as cook loss varied among different lots within temperature treatments, as noted by the standard errors, the differences were not significant. The physical condition of the crabs probably affected the amount of fluid lost, causing the observed variation within lots. Crabs which appeared to have just molted tended to exude less than crabs which were not molting. Other conditions, such as the amount of food in the crab's entrails may have affected the volume of cook loss.
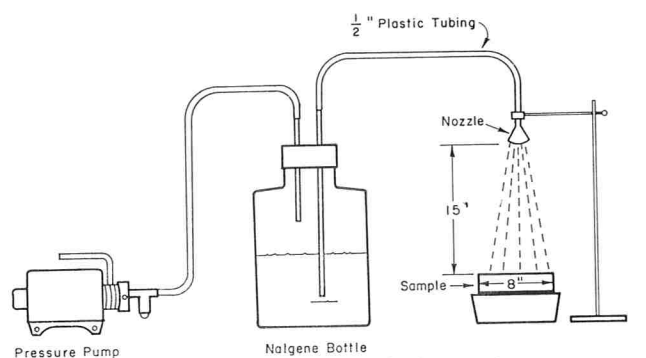
Figure 1. Schematic diagram of the rinsing apparatus.

TABLE 1. EFFECT OF COOKING TEMPERATURE ON COOK LOSS
OF BLUE CRABS

Temperature of steaming (°C)	Average sample weight (g)	Cook loss[a] (ml)	Cook loss (%)
100	2208.6	211.5 ± 40.3[b]	9.58[b]
121	2066.6	429.5 ± 40.3[b]	21.30[b]

[a]Expressed as the mean and standard error of 10 trials (lots).
[b]Significantly different at 0.01 level. Values for cook loss
were calculated from the cook loss and initial sample weight
data.

TABLE 2. PROXIMATE COMPOSITION OF COOKED MEAT
FROM THE BLUE CRAB

Component	%
Moisture	77.08
Protein	18.42
Fat	2.48
Ash	2.02

Crabs with missing appendages often exuded a white proteinaceous material from the resultant opening although this condition was minimal. The difference between cooking temperatures was highly significant at the 0.01 level. Differences in cooking conditions were minimized by adjusting the steam by-pass on the autoclave and the subsequent slow (5 min) release time of steam pressure. The increase in loss for the 121 C cook temperature was attributed to the increased temperature and pressure and the apparent stress on the component parts of the crab, especially the viscera. The higher temperature and pressure (15 psig) probably ruptured cells or cellular structure to a greater extent than the 100 C cook temperature causing the loss of more fluid. The greater severity of the higher temperature, higher pressure, and slightly longer cook time could also have caused more denaturation of proteinaceous material and the subsequent loss of fluid due to a reduction in water binding capacity.

The pH values indicated that the cook loss fluids were slightly acidic for both treatments (6.85 for 100 C, 6.86 for 121 C). The cook loss fluids were not significantly different in pH for the two cook temperatures. This indicated that cook temperature did not significantly change the acid concentration due to the release of amino or organic acids. This may be explained by the proteins in the solutions acting as strong buffers. The pH of whole, raw crabs was found to be 7.1 - 7.2 and it was found to be 8.0 for whole cooked crabs (cooked at 121 C for 10 min). This difference indicates that cooking the crabs caused changes in the pH which may be attributed to such factors as the release of organic acids or denatured proteins giving up bound cations. The rise in pH of the cooked crab to 8.0 may have been due to reduced buffering capacity of the heat denatured proteins.

The proximate composition of the cooked meat from blue crabs is presented in Table 2. These data were determined for use in establishing a basis for protein levels lost in the rinsing and dipping experiments. The amount of protein in the cook loss and the composite centrifuged fractions (solid and supernatant) of the cook loss are shown in Table 3. The values for protein reported in this study were based on an analysis for total nitrogen × 6.25. It was recognized that all of the nitrogenous material may not have been proteinaceous. The amount of protein in the supernatant of the cook loss from the 121 C cook was significantly higher than from the 100 C cook. The percent protein in the solids (centrifuge residue), however, showed an inverse relationship, the protein being slightly, but not significantly, higher in the solids from the 100 C than the 121 C cook. There were no significant differences among trials within the fractions, thus indicating that the sampling procedures were valid estimates of the population. The higher percentage of protein in the fluids from the 121 C cook was most likely related to the factors indicated as causing the greater cook loss for this treatment.

The amount of protein lost due to cooking was also expressed on the basis of the weight of the whole, raw crabs. Table 3 shows that the protein in the cook loss of the 121 C cook was significantly greater than the protein in the cook loss from the 100 C cook, when based on raw crab weight (mg protein/g crab). Thus, both percent cook loss and percent protein within the cook loss increased significantly for the higher temperature.

The data for protein losses due to tap water rinsing and NaCl dipping under pilot laboratory conditions are given in Table 4. The data for individual trials are presented to indicate the degree of variability, since significant differences were found among trial lots. The rinse water used for rinsing the meat from crabs steamed at 100 C had a larger percentage of protein in it than did the rinse water used for the

Table 3.   Effect of cooking temperatures on the amount of protein loss in various fractions of the cook loss fluid

Treatment (°C)	Sample fraction	Number[a]	Protein[b] (%)	Protein loss (mg) per g raw crab[c]
100	Total cook loss	28	3.1 ± 0.2[d]	2.9
	Supernatant	30	1.9 ± 0.2[e]	2.3
	Solids (dry residue)	16	74.4 ± 2.8[e]	0.6
121	Total cook loss	29	4.4 ± 0.2[d]	9.4
	Supernatant	30	3.3 ± 0.2[e]	6.9
	Solids (dry residue)	19	67.9 ± 2.6[e]	2.6

[a]All trials were done in triplicate, except the 121 C solids fraction was done in duplicate.
[b]Data are presented as mean and standard error of the mean.
[c]Calculated values.
[d]Significantly different at 0.01 level.
[e]Significantly different at 0.05 level.

Table 4.   Effect of cook temperature on the amount of protein loss during tap water rinsing and 8% NaCl dipping of blue crab meat

Cook temperature (°C)	Trial number and date[a]	Tap water rinse		8% NaCl dip	
		(%)[b]	Mg/g crab meat	(%)[b]	Mg/g crab meat
100	1 (Nov. '71)	0.17[c]	17.6	0.42[d]	43.4
	2 (Dec. '71)	0.51[d']	52.7	0.44[d]	45.5
	3 (Dec. '71)	0.72[d']	74.4	0.40[d]	41.3
	4 (Jan. '72)	0.19[c]	19.6	0.10[c]	10.3
	5 (Jan. '72)	0.22[c]	22.7	0.13[c]	13.4
	6 (Jan. '72)	0.26[c]	26.9	0.24[c]	24.8
	7 (Jan. '72)	0.14[c]	14.5	0.14[c]	14.5
	8 (Jan. '72)	0.18[c]	18.6	0.10[c]	10.3
	9 (Jan. '72)	0.18[c]	18.6	0.11[c]	11.4
	10 (Jan. '72)	0.18[c]	18.6	0.13[c]	13.4
	X̄	0.28 ± .02	24.8	0.22 ± .02	22.8[c]
121	1 (Nov. '71)	0.14[c]	14.5	.25[d]	25.8
	2 (Dec. '71)	0.37[d']	38.2	.20[d]	20.7
	3 (Dec. '71)	0.44[d']	45.5	.25[d]	25.8
	4 (Jan. '72)	0.18[c]	18.6	.09[c]	9.3
	5 (Jan. '72)	0.21[c]	21.7	.11[c]	11.4
	6 (Jan. '72)	0.17[c]	17.6	.11[c]	11.4
	7 (Jan. '72)	0.20[c]	20.7	.10[c]	10.3
	8 (Jan. '72)	0.20[c]	20.7	.11[c]	11.4
	9 (Jan. '72)	0.15[c]	15.5	.09[c]	9.3
	10 (Jan. '72)	0.12[c]	12.4	.10[c]	10.3
	X̄	0.22 ± .02	22.5	0.14 ± .02	14.6[d]

[a]Each trial was conducted in triplicate.
[b]Each sample consisted of 150 g crab meat rinsed with 1,550 ml of tap water or dipped in 1,550 ml 8% NaCl. Data are expressed as mean and standard error of the mean. Means which have different suffixes (c, d, d') were significantly different. d' Designation indicates significance at the 0.01 level, whereas differences at the 0.05 level are indicated by c and d.

crabs steamed at 121 C. This difference in protein of the tap water rinse was not significant between temperatures of steaming but there was a highly significant difference (0.01 level) among trials. The percent protein for each trial ranged from 0.12 to 0.26%, except for trials 2 and 3. The percent protein in the rinse water of these two trials was significantly higher, ranging from 0.37 to 0.72%. The crabs used in these two trials were the only crabs harvested during the month of December, crabs for the other trials being harvested almost a month before or after these trials. The physiological condition of the crab is known to be cyclic. Immediately after molting, the

crab's body expands in size by the copius intake of water with a consequent increase in the volume of the body fluids (9). Although molting was not obvious, data in Table 4 suggest that a major physiological change had taken place. On the basis of previously reported data (12), it was concluded that the higher losses from the December-harvested crabs were due to a seasonal variation in the physiological condition of the crab, not necessarily molting, with a consequent difference in the meat. Therefore, the total proteinaceous losses might be expected to be greater during these periods.

By calculations, similar to those used to derive the

results for Table 3 for the cook loss data, the total amount of protein lost in each rinse can be determined, as well as the amount of protein lost per unit weight of crab meat rinsed. For the crabs cooked at 100 C, the meat lost 28.4 mg protein/g meat rinsed. The meat from the crabs cooked at 121 C lost 22.5 mg protein/g meat rinsed. On the basis of the analysis of steamed and picked crab meat (18.42% protein, Table 2), these losses are equivalent to 15.2% of the total protein for the 100 C cooked crabs and 12.6% of the total protein for the 121 C cook crabs. The data indicate that the cooking procedure using the higher temperature removed more of the soluble protein than occurred with the 100 C cook. Thus, if the protein was not lost during the cook, it was removed during the rinse. This would account for the greater amount of protein in the rinse solution of the 100 C cooked crab meat. The pH values of the rinse waters from the two treatments were not significant (6.36 for 100 C and 6.59 for 121 C) but were slightly lower than the pH of the cook loss fluids.

Crabs steamed at 100 C lost significantly greater amounts of protein in the salt brine dipping process than those steamed at 121 C. This followed a similar trend but to a greater degree than for the tap water rinse. There was a significant difference among the trials as illustrated in Table 4, showing that trials 1, 2, and 3 had the greatest protein loss within both treatments. As stated for the tap water rinsed samples, these trials were made using crabs harvested in late fall and early winter. The pH values for the salt dip solution after dipping the crab meat were not significantly different between the two cook temperatures (6.73 for 100 C and 6.51 for 121 C). The pH values for the cook loss fluids, tap water rinse, and salt brine dip were all lower than the cooked crab meat (pH 8.0). This indicated the removal of primarily acidic components from the crab meat under these simulated processing conditions.

The total amount of protein lost in the salt brine dip was less than the amount lost in the tap water rinse but the differences were greater for the latter technique. The tap water rinse utilized a pressurized spray, which contributed some physical action to the rinsing process; whereas, in the dipping process, no agitation other than that involved in lowering and raising the product into the solution was involved. The amount of protein leached out of the crab meat by the salt brine dipping operation was similarly important. The 22.8 mg protein lost per gram of crab meat which had been cooked at 100 C amounted to a loss of 11.2% of the total protein of the dipped crab meat and the 14.6 mg protein lost per gram of crab meat which had been cooked at 121 C was equivalent to 7.3% of the total protein.

The total protein content of the crab meat was substantially reduced by the rinsing and dipping treatments. These losses are major considerations in nutritive value as well as pollutional load strength for crab processing plants. When these losses are combined with the amounts lost during the cooking operation, the total represents a sizable fraction of the total protein of the crab. This investigation did not attempt to establish specific acceptable levels of protein which might be lost during processing. The results present rather conservative levels of protein loss under laboratory pilot conditions as compared to commercial operations. It appears that the losses observed in this study indicate the need for developing crab processing systems which will reduce the loss of protein.

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CONDITION OF COLIFORM ORGANISMS INFLUENCING RECOVERY OF SUBCULTURES ON SELECTIVE MEDIA[1]

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Abstract

The effects of stress of coliform bacteria resulting from exposure to heat, radiation, or sodium chloride on behavior of the progeny were studied. After exposure to stress and subsequent growth on plating media, colonies were picked by random design and grown in nutrient broth for further comparison of their ability to form colonies on plate count and violet red bile agar. After 6 hr in nutrient broth, average counts on violet red bile agar were less than half those obtained with plate count agar. Sensitivity to the selective medium was lost by repeated transfer and growth in nutrient broth or by repeated picking from the selective medium and subculture of colonies.

Cultures with moderate sensitivity to violet red bile agar were obtained from raw sewage through picking of colonies from plate count agar. Attempts to obtain a stable sensitive strain through selective enrichment were unsuccessful. A laboratory strain of *Escherichia coli*, with extreme sensitivity to violet red bile agar, however, was used to determine that tolerance was acquired by stepwise adaptation to a selective medium. Occurrence of sensitive coliform cells in nature indicates their potential importance in tests for indicator organisms of public health significance.

Coliform bacteria associated with food handling systems are of interest as indicators of organisms of public health significance. Bacteria in the microenvironment of food handling equipment are commonly subjected to stress and thus become more sensitive to their growth environment (2, 4, 6-10, 12). Some are apparently "injured." Injured cells are sensitive to surface active agents in selective media normally useful for enumerating coliform bacteria (4, 11).

It has been tacitly assumed that stressed cells on subsequent recovery and growth gave rise to normal cells. Simple methods for proving this assumption, however, were not available. Purposes of research reported here were to examine the hypothesis that stress treatments influence subsequent cultures and to study conditions required for the progeny to regain normal resistance to selective components of media.

Methods

Cultures

Enterobacter aerogenes and one strain of *Escherichia coli*

were from the departmental culture collection. A strain of *E. coli* with extreme sensitivity to selective media for coliform determinations was obtained from the Department of Microbiology, University of Nebraska, Lincoln. The cultures were propagated in nutrient broth (NB; Difco) at 32 C for 18-24 hr and held at 3-5 C for storage.

Media

The medium which served as a standard for comparison was plate count agar (PCA; BBL or Difco). The selective medium was violet red bile agar (VRBA; BBL or Difco). Brilliant green lactose bile broth (BGLBB; Difco) was used to determine gas production and "presumptive evidence" of coliform organisms (1). Minimal agar (MA) consisted of $NH_4H_2PO_4$, 0.3%; K_2HPO_4, 0.2%; iron as $FeSO_4 \cdot 7H_2O$, 0.5 ppm; $MgSO_4 \cdot 7H_2O$, 0.05%; glucose, 0.3%; agar, 1.5%. The pH was adjusted to 7.0 by the addition of 5N KOH. Solutions of glucose and $MgSO_4$ were autoclaved separately and added to the medium before plating.

Plating and enumeration

Plating and counting procedures were those recommended by the American Public Health Association (1). The difference between the PCA count and the VRBA count was attributed to injured or to sensitive cells. When colonies were picked for further study it was by random design from countable plates. They were then grown for 6 hr in NB at 32 C and stored at 2 C until plate counts were made—the elapsed time never exceeded 24 hr.

Procdure for stress of cells

To obtain heat stressed cells, cultures were heated without agitation at 60 or 65 C according to a previously described method (4). Obtaining stress by exposure to 5% NaCl has also been described (4). Radiation stress was by exposure to cobalt-60 as described by Tiwari and Maxcy (13). Each process was repeated at least three times after adjustment of conditions to obtain approximately 95-99% kill of the original culture.

Enrichment of sensitive cultures

Procedures for selective enrichment were based on the work of Lederberg and Zinder (3). Growing cells are sensitive to penicillin. After destruction of penicillin by penicillinase, previously inactive cells can be made to grow.

Adaptation of a sensitive culture

A sensitive strain of *E. coli* was grown in progressive, challenging concentrations of quaternary ammonium compound in NB according to a procedure described by Maxcy et al. (5) to obtain a resistant strain.

Results

Injury and effect on subsequent generations

To determine if subcultures remained sensitive to selective media, colonies were picked from PCA and subcultured in NB. Presumably, if subcultures re-

TABLE 1. THE EFFECT OF VARIOUS FORMS OF INJURY ON THE SENSITIVITY OF SUBCULTURES TO VIOLET RED BILE AGAR

Method of injury	Organism	Number of isolates observed	Per cent injured cells		
			Mean	Range	Standard deviation
Heat	E. coli	78	58	16-99	5
Heat	E. aerogenes	68	34	0-70	—
Radiation	E. coli	78	69	0-98	25
NaCl	E. coli	72	57	15-97	21

tained sensitivity, injury was to a genetically transmissible trait. Three systems of cell stress were used to study the response of E. coli. Results were expressed in terms of per cent of injured cells, which was the difference between the PCA and VRBA counts divided by the PCA count with the quotient multiplied by 100. From a study of 78 subcultures of heat injured E. coli, for example, the mean per cent of cells recovered was 58, thus indicating 42% of the cells had altered characteristics compared to the parent culture. A summary of the results with subcultures is in Table 1. The mean per cent of injured cells for the various treatments and bacteria was 34-69. These results are in agreement with results obtained with the parent culture as previously reported (4). Thus the sensitive characteristic persisted through a subculture indicating the phenomenon was genetically related. Results with E. aerogenes were similar to those with E. coli as judged by data pertaining to heat stress.

Isolates showing the greatest sensitivity to VRBA were studied further in an attempt to obtain strains with a high degree of sensitivity. From the most sensitive 6-hr culture, an 18-hr subculture in NB was prepared and subjected to stress. Platings were made and 30 colonies were picked for 6-hr subcultures in NB. Comparative platings of the individual subcultures were then made on PCA and VRBA. The results indicated that the mean per cent of injured cells was similar to the original culture before the stress treatment. Thus it was not possible to obtain a further increased proportion of sensitive cells.

Stressed cells were treated with various concentrations of penicillin and for various times after which penicillinase was added to inactivate the penicillin. This system to destroy growing cells did not provide the hoped for increase in proportion of sensitive cells in the population.

To obtain data on the relative stability of sensitivity in cultures after stress treatments, subcultures were made in NB. The most sensitive cultures reverted to the normal resistance of the original stock culture within 6 daily transfers.

To determine if there was a difference in the proportion of sensitive cells from colonies growing on VRBA and on PCA, isolates were obtained from VRBA plates made to evaluate stress treatments.

Subculture of these isolates for 6 hr in NB followed by plating on VRBA and PCA gave results that showed the cells had the same sensitivity toward the selective medium as when colonies taken from PCA were similarly subcultured and plated.

Cultures treated to obtain stressed cells developed various colony sizes when grown on VRBA. There was no apparent difference between small and large colonies, however, in comparative sensitivity to VRBA.

With this system of observation it was apparent that the type of injury was similar irrespective of the method of stress. Perhaps the apparent similarity resulted from the arbitrary adjustment of the stress treatment to get a 95-99% kill of the test culture.

Occurrence of VRBA-sensitive strains in nature

Samples of mixed raw sewage, representing effluent from most of the city of Lincoln, Nebraska (population approximately 150,000), were plated on PCA. Ten colonies from each of three countable plates were picked by random design into NB and into BGLBB. Those colonies producing gas in BGLBB were considered coliform organisms and observed further by using the inoculum that had been put into NB. Comparative counts from the NB were made with duplicate plates using PCA and VRBA. When the VRBA count was less than 50% of the PCA count and a repetition of the plating also indicated less than 50%, the isolate was arbitrarily considered sensitive to VRBA. From 31 samples of sewage, 102 coliforms were isolated and 6 of these were sensitive to VRBA.

Comparisons of VRBA and PCA counts on the sewage samples indicated that 9.9% of the total population was coliforms. Isolates from PCA counts were obtained by random design, and results showed 14% of the total population to be coliforms as judged by their ability to produce gas in BGLBB. A comparison of these methods indicated only 71% of the coliforms were enumerated by the VRBA method.

Gain and loss of VRBA sensitivity

A particularly sensitive strain of E. coli was used to determine conditions contributing to development of resistance to VRBA. A typical count for an 18-hr culture from NB was 3.2×10^8 on PCA and 6.0×10^2 on VRBA. After subculturing seven times on

VRBA, a typical count from an 18-hr culture of NB was 3.1×10^8 on PCA and 3.0×10^7 on VRBA. The adaptation process was stepwise. Counts on MA were not significantly different from those on PCA.

When the strain of *E. coli* particularly sensitive to VRBA was subcultured for 10-12 days by serial transfer in BGLBB, there was a marked loss in sensitivity to VRBA. A similar reduction in sensitivity was attained through stepwise adaptation of the sensitive parent culture to 28 mg of quaternary ammonium compound per liter of NB. Altered sensitivity to VRBA was maintained even after 5 subcultures in NB without quaternary ammonium compound.

DISCUSSION

Cell stress and altered recovery on various media is well recognized. While the mechanism is not understood, it most often has been attributed to changes in nutritional requirements, because richer media commonly yielded more cells. Auxotrophic mutants, however, have not been found. Furthermore, in the work reported here attempts to increase the proportion of sensitive cells in a culture were unsuccessful.

The effect of stress persisted through subculture, therefore, indicating a genetic relation. Conditions for isolating these strains with sensitivity as a stable factor, however, remained obscure. Since the sensitive strain from the Department of Microbiology acted as a mutant, it may be projected from our data that the sensitivity is genetically related and not nutritionally dependent. Classical methods for isolating auxotrophic cultures, as used by Postgate and Hunter (8) and Russell and Harris (9), therefore, would not be applicable. Since the sensitive culture acquired tolerance for VRBA through subculture on VRBA, growth in BGLBB, and growth in the presence of a quaternary ammonium compound, sensitivity apparently is related to surface activity.

Sensitive strains occur in nature therefore emphasizing the problem of cell stress and recovery on selective media. Sewage should not be considered an extremely adverse environment; a greater proportion of sensitive cells, therefore, might be expected from other more adverse environments. There are numerous conditions in the food industry where stress is common, and cells would remain in an adverse environment. The problem of stress and recovery of stressed cells on selective media, therefore, continues to be of interest and of public health significance.

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PASTEURIZATION OF GRADE "A" MILK AND MILK PRODUCTS BY STEAM INJECTION

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ABSTRACT

Steam injection systems are in common use in some parts of the United States. They have not been recognized, however, as a satisfactory method for pasteurizing milk and milk products because of possible poor mixing of steam and product, vapor formation in the holding tube, and inadequate public health controls. Recent research has solved these problems and steam injectors may be used to pasteurize milk products when the steam and product flows are isolated from the pressure fluctuations in the injection chamber, when no vapor is formed in the holding tube, and when non-condensable gases are eliminated from the steam supply. The injection chamber is isolated by installing supplementary orifices of a proper size at the injector ports to maintain a product pressure drop of 10 psi across the injector. Vapor formation is prevented by maintaining a holding tube pressure 10 psi above the boiling pressure of the liquid product. Non-condensable gases are eliminated from the steam supply by installing an approved deaerator in the boiler system.

Ultra-High Temperature (UHT) processes by plate heat exchange have been identified for milk and milk products (9); however, the commercial application of these processes has been limited to cream and flavored products that are relatively heat stable. During pasteurization, the total heat treatment given the product is the sum of that received during heating, holding, and cooling, and in plate heat exchangers, the heating and cooling phases represent a significant portion of the total heat treatment. Conversely, in steam injection systems, heating and cooling are virtually instantaneous, and as a result, the total heat treatment is only slightly greater than that received in the holding tube. Because of this, steam injection systems appear useful in minimizing organoleptic damage caused by the high temperatures of UHT processes.

Steam injection systems are used routinely in southern and central parts of the United States for flavor control of milk products, and adequate controls are supplied with these systems to prevent dilution of product with water. Such systems have not been used for pasteurization, however, because of possible inadequate mixing of steam and product (7), reduced residence times in the holding tube due to vapor formation, and inadequate controls for the instantaneous temperature drop that occurs with a loss of steam (8).

Recent research has yielded solutions to these problems. Temperature variations in product leaving the injector are negligible when the steam and product flows are isolated from the pressure fluctuations in the injection chamber (11). Residence times in the holding tube are constant when the pressure of product in the holding tube is held 10 psi above the boiling pressure of the liquid (3); and adequate controls have been developed for steam injection systems (12). These research accomplishments have enabled the identification of satisfactory processes for pasteurization of milk and milk products by steam injection.

TIME-TEMPERATURE STANDARDS

The recommended time-temperature standards are identical to those specified for ultra-high temperature pasteurization by plate heat exchange (9). These are: (a) 1 sec hold at 191 F, (b) 0.5 sec hold at 194 F, (c) 0.1 sec hold at 201 F, (d) 0.05 sec hold at 204 F, and (e) 0.01 sec hold at 212 F.

These combinations of time and temperature are minimum requirements, and actual processing conditions may exceed these requirements in either time or temperature. The time and temperatures are applicable to all milk and milk products which are now being pasteurized in plate-type pasteurizers (whole milk including Vitamin D and fortified, skim milk, low fat milk, chocolate milk and drink, cream, frozen dessert mix, eggnog, and concentrated milk).

EQUIPMENT

The equipment is similar to that now used for conventional pasteurization and flavor control, where a plate heat exchanger, holding tube, and flow diversion valve are used to pasteurize the product before steam injection. For steam injection pasteurization, however, the product is preheated in a plate heat exchanger, heated to pasteurization temperature in the steam injector, pasteurized in the holding tube, precooled in the vacuum tank, cooled to storage temperature by the plate heat exchanger, and then controlled by the flow diversion valve (Fig. 1).

INSTALLING THE INJECTOR

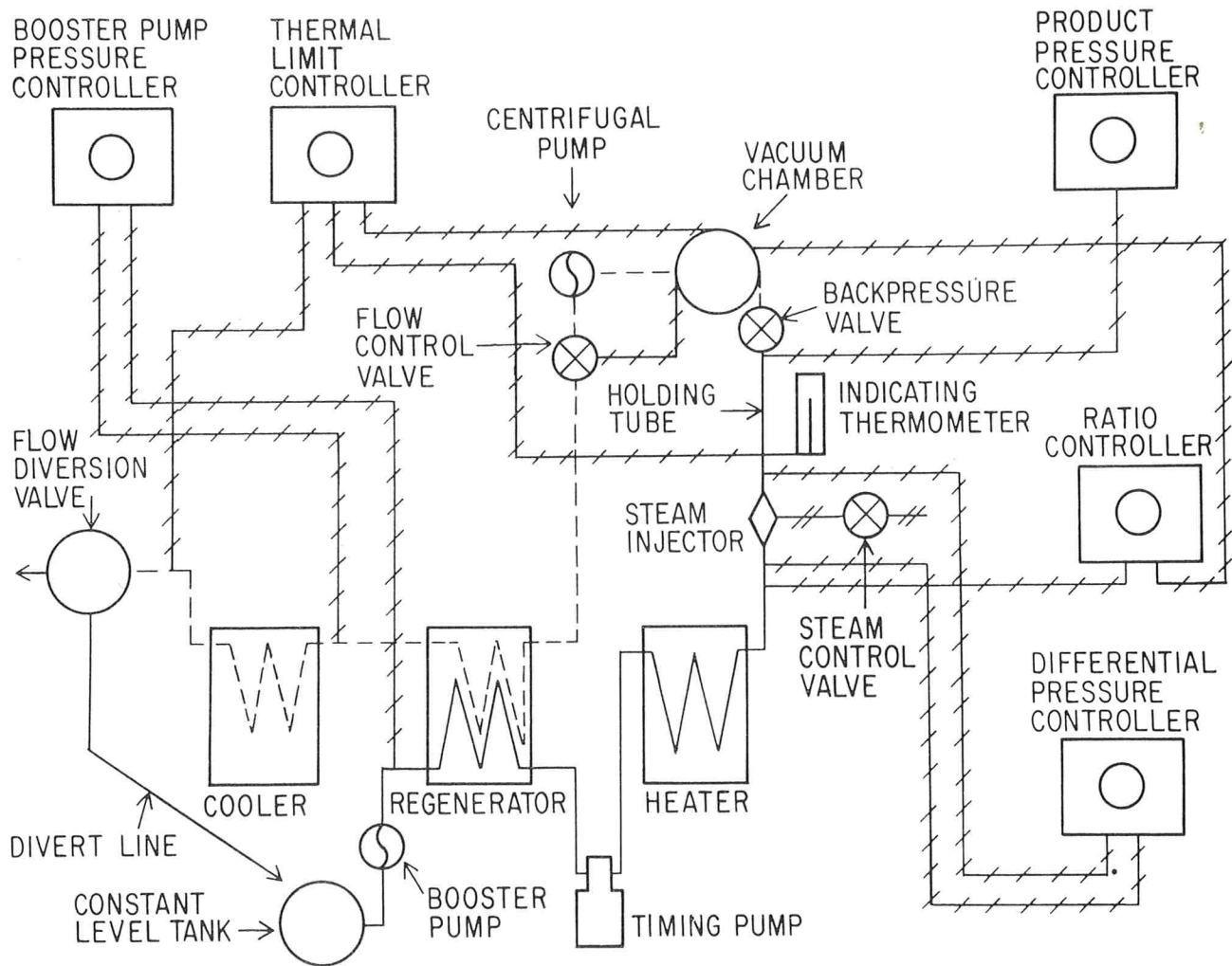Of all the problems associated with steam injectors,

Figure 1.   Flow diagram and controls for steam injection pasteurizers.

inadequate mixing of steam and product is the most serious. Unless the injector is installed properly, temperature fluctuations of $\pm$ 28 F may occur (11), and these fluctuations may persist for significant distances into the holding tube (7, 11). Under some conditions, the temperature variations can be large enough to prevent normal operation of the temperature controller. Roberts and Dill (10) reported this problem in 1962 and minimized the temperature variations by installing an orifice at the injector exit.

To ensure proper operation, steam and product flows must be isolated from the pressure fluctuations in the injection chamber (11). This is done by placing supplementary orifices at the product inlet and heated product outlet ports of the steam injector. A pressure drop of 10 psi across the injector is needed to ensure proper operation (11). Since this pressure drop will vary with flow rate and product, a differential pressure control diverts flow whenever the pressure drop across the injector is less than 10 psi. Diagrams of several steam injectors are available elsewhere (6, 11).

Vapor formation in the holding tube is prevented by maintaining injection pressure 15 psi higher than the boiling pressure of the liquid in the holding tube (3). Since the pressure drop across the orifice at the injector exit is at least 5 psi, the above requirement is met by maintaining product pressure in the holding tube at least 10 psi above the boiling pressure of the liquid in the holding tube (11). An absolute pressure control diverts flow when this condition is not met.

TIMING THE HOLDING TUBE

Since the flow of some dairy products is characterized as laminar and under these conditions the residence time of the fastest product can never be less than one-half that of the average of all particles (2), holding time is computed in a manner similar to that developed for UHT pasteurization by plate heat exchange (1). The procedure was modified to reflect a 12% flow increase by steam injection. Not all processes will require the injection of this much steam,

so a small margin of safety exists for most processes. A steam addition rate of 12% (12 lb. of steam per 100 lb. of product) yields a temperature rise across the injector of 120 F, and is adequate to cover most operations.

CONTROLS

Most UHT pasteurizers have the flow diversion valve located at the end of the cooling section. This obviates two major problems. When the flow diversion valve is located at the end of the holding tube, it diverts unpasteurized product to the raw product supply tank, and stops flow of product to the pasteurized side of the regenerator. Any product at a temperature above 212 F, however, will boil when diverted to atmospheric pressure. From the plant operator's viewpoint, stopping the flow of product at the inlet to the pasteurized side of the regenerator causes more serious problems. At UHT temperatures, product quickly burns on to the heat exchange surfaces, and any significant flow stoppage requires a shutdown and a cleaning operation. The fluid in the raw product side of the regenerator continues to recirculate without cooling, but even a brief period of diverted flow can cause extensive flavor damage to the recirculating product (4).

With steam injection systems, there is the additional problem of control response times. A complete loss of steam pressure causes a precipitous drop in temperature, and conventional controls do not have the speed of response required to prevent the forward flow of unpasteurized product (8). However, by locating the flow diversion valve at the end of the cooling section rather than at the end of the holding tube, the conventional controls are sufficiently fast to prevent the forward flow of unpasteurized product after a complete loss of steam pressure. Consequently, for steam injection systems, the recommended location for the flow diversion valve is at the end of the cooling section. The controls required for steam injection systems are as follows:

Temperature controllers. The safety thermal limit recorder-controller controls the flow diversion valve to prevent mixing of raw and pasteurized products, and it can do this unassisted, only when the flow diversion valve is located at the end of the holding tube. When there is a diversion with the flow diversion valve located after the cooler, the vacuum chamber, the pasteurized side of the regenerator, and the cooler become contaminated with raw product, and forward flow must not occur until all product surfaces downstream from the holding tube have been sanitized. This is accomplished by installing two additional temperature controllers in the system. The sensing element of the safety thermal limit recorder-controller is installed in the product at the beginning of the holding tube. The sensing element of one auxiliary controller is installed in the steam vapors in the top of the vacuum chamber, and the other is installed in the product at the common port of the flow diversion valve. The three temperature controllers are interwired to prevent forward flow until the three temperature sensing elements have been exposed to fluid at pasteurization temperature, continuously and simultaneously for pasteurization time. This ensures that all product surfaces between the three sensing elements have been sanitized. To do this, the cooling water must be turned off and this is usually done automatically.

When the pasteurized lines have been sanitized by the above procedure, the sensing elements at the vacuum chamber and the flow-diversion valve are automatically dropped from the control circuit, the cooling water is turned on, and forward flow is permitted for as long as pasteurization requirements are met in the holding tube. If the temperature of product in the holding tube drops below that required for pasteurization, the flow-diversion valve is moved to the divert position, the sensing elements at the vacuum chamber and the flow-diversion valve are automatically returned to the control circuit, and forward flow is prevented until all of the product surfaces downstream from the holding tube have been sanitized.

Differential pressure controller. A differential pressure control is installed across the steam injector with sensors in the product flows entering and leaving the injector (Fig. 1). When the pressure differential across the injector is less than 10 psi, the controller moves the flow diversion valve to the divert position. Forward flow is prevented until the product surfaces downstream from the holding tube have been sanitized as described above.

Absolute pressure controller. An absolute pressure control is installed in the holding tube and is adjusted to move the flow diversion valve to the divert position whenever product pressure is less than 10 psi above the boiling pressure of the liquid in the holding tube (Fig. 1). Forward flow is prevented until the product surfaces downstream from the holding tube have been sanitized as described above.

Ratio controller. As with all steam injection systems, a ratio controller is required to prevent dilution of product (Fig. 1). The ratio controller and the steam supply will be interlocked in the same manner as for the high-temperature, short-time processes.

STEAM SUPPLY

The steam supply must be the same as that speci-

fied for existing flavor control systems (13), with one additional provision: non-condensable gases must be removed. Non-condensable gases in the steam supply are injected into the product, and, although they are removed in the vacuum tank, they reduce the effectiveness of pasteurization.

Using a glass holding tube, Peterson and Jordan (6) photographed non-condensed gases after steam injection. Since the non-condensable gases enter the product downstream from the metering pump, they displace product in the holding tube and cause a reduction in holding time. Non-condensed gases also reduce the effectiveness of the mixing process between steam and product (3, 5, 11).

To prevent the introduction of non-condensable gases through the steam injector, the steam boiler must be supplied with a deaerator and must be installed in accordance with the requirements of a qualified boiler feed water treatment authority.

When used in conjunction with approved and properly operated ancillary equipment, and when installed, and operated as described herein, steam injectors are acceptable for pasteurization of milk and milk products under that portion of definition S of the Grade "A" Pasteurized Milk Ordinance (13) which provides for other pasteurization processes recognized by the Food and Drug Administration to be equally efficient. More detailed information on methods and procedures for the inspection of equipment as well as the necessary wiring diagrams used for pasteurization by steam injection will be provided upon request.

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AFLATOXIN PRODUCED BY *ASPERGILLUS PARASITICUS* WHEN INCUBATED IN THE PRESENCE OF DIFFERENT GASES[1]

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ABSTRACT

The influence of atmospheric gases on aflatoxin formation by *Aspergillus parasiticus* was investigated using a chemically defined medium and controlled environments in a fermenter. Maximal yield of aflatoxin was obtained under fermentation conditions lacking agitation or sparging of air. Increasing the rate of aeration enhanced glucose utilization and acid formation, but reduced toxin production. Replacement of air by various mixtures of O_2 and CO_2, or O_2 and N_2 suppressed toxin formation. Increasing proportions of CO_2 or N_2 in the atmosphere enhanced their inhibitory effect on aflatoxin formation, and complete inhibition of toxin synthesis occurred in atmospheres of 100% CO_2 or 100% N_2. Synthesis of toxin was suppressed more by a high concentration of CO_2 than of N_2. The optimal condition for toxin formation was quiescent incubation. Suppression of toxin formation can be achieved by introducing high concentrations of CO_2 or N_2 into the environment.

Molds in the genus *Aspergillus*, more specifically *Aspergillus flavus* and *Aspergillus parasiticus*, can produce aflatoxin which is acutely toxic or carcinogenic to experimental animals. Because of its biological effects and its occasional appearance as a contaminant of agricultural commodities, including foods, this fungal metabolite has been widely investigated (11, 21).

Growth of storage fungi such as the aspergilli in natural substrates is dependent not only on availability of moisture and favorable temperature, but also on atmospheric conditions surrounding the substrate (15). The influence of atmospheric gases on germination, growth, and metabolic activities of molds was discussed by Tabak and Cooke (18). Use of controlled atmospheric environments to retard microbial activities has been applied practically to store some feed and food products. Bampton (1) indicated that storage conditions which limit growth of *A. flavus* should keep a food-stuff aflatoxin-free.

Several reports have appeared on the effects of various atmospheric conditions on aflatoxin formation in agricultural commodities (6, 9, 14). However, most such studies have not been done in a chemically defined liquid medium and hence have been subject to variable effects caused by the different substrates that were used. It was believed that atmospheric conditions could be more effectively controlled by using a liquid medium in a fermenter than when a solid substrate was used in which the gas or gases might not be exchanged uniformly. In addition, use of a liquid medium in a fermenter provides an opportunity to study toxin formation by the resting mycelium under conditions unfavorable for germination of spores and growth of the mold.

In this study a toxin-producing strain of *A. parasiticus* was cultured in a liquid medium in a fermenter with air (with and without sparging) and with other controlled gaseous environments. Effects of these conditions on aflatoxin synthesis were measured to determine (a) how to obtain a high yield of aflatoxin, and (b) if a controlled gaseous atmosphere could be employed to reduce production of aflatoxin. Results of the experiments are reported in this communication.

MATERIALS AND METHODS

Organism

Aspergillus parasiticus NRRL 2999, a toxigenic strain, was obtained from the Northern Regional Research Laboratory, Peoria, Illinois. The mold was grown on mycological agar (Difco) slants at 28 C for 6-8 days before use.

Medium

A glucose-salts-amino acids broth formulated in our laboratory was used as the fermentation medium. The medium was composed on the basis of reports by Mateles and Adye (12), Davis et al. (5), and Lee et al. (10) and contained (per liter) 50 g glucose, 6 g $(NH_4)SO_4$, 5 g KH_2PO_4, 6.4 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g HCl, 2 g glycine, 2 g glutamic acid, 10 mg $FeSO_4 \cdot 7H_2O$, 5 mg $ZnSO_4 \cdot 7H_2O$, and 1 mg $MnSO_4 \cdot H_2O$. The medium was prepared by aseptically adding the sterile glucose solution to the sterile salts-amino acids solution in the fermenter after both solutions were cooled. The pH of the medium was 6.4 to 6.5.

Cultural conditions and fermentations

Fermentations using various gaseous environments were done as follows. A 1-liter Mini-Ferm fermenter equipped with an automatic temperature controller and a magnetic stirrer (model M-1000, Fermentation Design, Inc., Allentown, Pennsylvania) was used for the experiments. Each experiment was conducted with 500 ml of medium. The glucose-free salts-amino acids solution (450 ml) was placed in the fermenter jar and then jar and solution were sterilized in an autoclave. Glucose (50% solution) was autoclaved separately and then aseptically added to the salts-amino acids

solution to provide a final concentration of 5% sugar. Five milliliters of a spore suspension (7-8 $\times$ 10^6 spores/ml) were added to the medium and the fermentation was conducted at 28 C for up to 6 days. The following gaseous environments were tested for their effects on aflatoxin formation when the spore suspension served as inoculum: air with and without sparging, mixtures of O_2 and CO_2, and mixtures of O_2 and N_2.

In other experiments, the mycelium of *A. parasiticus* served as the inoculum. The mycelium was prepared by incubating 2 ml of the spore suspension with 100 ml glucose-salts-amino acids medium in a 500-ml Erlenmeyer flask at 28 C for 3 days. After the mycelium was harvested from broth by filtration, it was washed with cold sterile distilled water. Two and one-half grams of mycelium (wet weight) served as inoculum for each experiment and incubation was at 28 C for 3 days. When the mycelium served as inoculum, the following gaseous environments were used: air without sparging, 100% CO_2, 100% N_2, and mixtures of O_2 and N_2.

Each atmosphere was maintained by sparging air (2 different rates), a pure gas, or a mixture of gases through a filter into the culture medium at a rate of 0.5 ml gas/ml of medium/min, except for the static culture. One experiment utilized 1.0 ml air/ml of medium/min. The gaseous effluent from the fermenter was first exhausted through a filter packed with glass wool, and then was bubbled into a 0.01% $CaOCl_2$ solution. The stirrer rate was 120 rev/min, except when static incubation was used.

Analysis of fermentation broth

Approximately 30 ml of medium were removed from the fermenter after 3, 5, and 6 days of incubation for determination of pH, residual glucose content, and aflatoxin concentration. Glucose was determined by the anthrone test (19). Aflatoxin was measured as described below.

Aflatoxin determination

Twenty milliliters of broth were extracted with 40 ml chloroform; the extraction was repeated three times using a separatory funnel. The mycelium (Table 4) was harvested from broth by filtration and then was washed with cold distilled water. The mycelial mat was weighed to determine mold growth and then was blended with 50 ml chloroform, 100 ml methanol, and 40 ml water in a Waring blendor for a few minutes. Fifty milliliters of chloroform and 50 ml of water were then added to the mixture and it was blended again. After filtration, chloroform was separated from the mixture in a separatory funnel. The methanol-water mixture was then extracted twice with chloroform (17). Chloroform extracts from either broth or mycelium then were concentrated for aflatoxin analysis. Concentration of aflatoxin was determined by procedures described by Shih and Marth (16). Thin layer chromatographic plates were developed with chloroform:methanol:water (98:1:1, v/v/v).

RESULTS

Rate of aeration and aflatoxin formation

The influence of various rates of aeration on aflatoxin formation by *A. parasiticus* is shown in Table 1. The greatest concentration of aflatoxin was produced by the culture incubated in the fermenter without agitation or sparging of air through the medium. Aflatoxin production decreased when the culture was aerated, although the amount of toxin after 6 days was slightly higher with a aeration rate

of 1.0 ml air/ml/min than with one of 0.5 ml air/ml/min. Increasing the rate of aeration increased glucose utilization and acid production. These data do not clarify why more toxin was formed initially when the lower rates of aeration were used and after 6 days with the higher rate of aeration.

Various levels of O_2 and CO_2 and aflatoxin production

The highest concentration of toxin produced with variable amounts of O_2 and CO_2 was present in the broth when the fermentation was conducted using an atmosphere composed of 9 parts O_2 and 1 part CO_2 (Table 2). Less aflatoxin was produced when the proportion of CO_2 to O_2 was increased. Negligible amounts of toxin appeared when the mold grew in an environment of 90% CO_2 and 10% O_2. It is evident that increasing the concentration of CO_2 diminished production of aflatoxin. Acid production and glucose uptake also were reduced as the proportion of CO_2 in the atmosphere increased.

Various levels of O_2 and N_2 and aflatoxin production

Increasing the N_2 and decreasing the O_2 content of the atmosphere reduced aflatoxin formation, acid production, and glucose utilization (Table 3). Although the effect of N_2 on these activities followed a pattern similar to that observed for CO_2, it was less profound than that of CO_2. It was also noted that various levels of O_2 and N_2 were similar in their inhibition of toxin formation, whereas some differences existed when similar concentrations of O_2 and CO_2 were used.

Formation of aflatoxin in broth inoculated with mycelium

To eliminate the problem of inhibiting or retarding spore germination in the absence of O_2 (19), 3-day old mycelium also was used to study formation of aflatoxin in various gaseous environments including an atmosphere unfavorable for spore germination and mold growth. Results are in Table 4. The stationary culture produced the greatest concentration of aflatoxin, whereas mycelium incubated in atmospheres of 100% CO_2 or 100% N_2 essentially failed to form aflatoxin. Addition of O_2 to a N_2 (1:9 v/v) environment prompted the mycelium to resume toxin formation, and a further increase in the amount of O_2 (9:1 v/v; O_2:N_2) further enhanced toxin formation.

The extent of mycelial growth depended on the concentration of O_2 present; no growth occurred when the mycelium was incubated in atmospheres of 100% CO_2 or 100% N_2. These results demonstrate further that maximal aflatoxin formation is associated neither with maximal concentrations of O_2 present nor with maximal mycelial growth. Formation of greatest concentrations of aflatoxin in the static culture sug-

TABLE 1. AFLATOXIN FORMATION IN A LIQUID MEDIUM INOCULATED WITH SPORES OF *Aspergillus parasiticus* AND AERATED AT DIFFERENT RATES

| | Static culture, incubated for days | | | Aerated cultures, incubated for days | | | | | |
| | | | | 0.5 ml air/ml[a]/min | | | 1.0 ml air/ml[a]/min | | |
	3	5	6	3	5	6	3	5	6
Aflatoxins[b]				(μg/500 ml)					
B_1	12	1200	4113	123	385	368	53	120	488
B_2	5	75	325	14	63	65	10	10	75
G_1	9	925	3000	97	325	340	103	150	336
G_2	3	63	225	8	40	48	8	20	43
Total	29	2263	7663	242	813	821	174	300	942
pH	5.8	4.8	4.1	5.4	4.5	3.9	4.9	3.8	3.6
Residual glucose (%)	3.10	2.47	1.92	3.70	2.84	2.36	2.46	1.70	1.00

[a]Medium volume.
[b]Aflatoxins in broth.

TABLE 2. AFLATOXIN FORMATION IN A LIQUID MEDIUM INOCULATED WITH SPORES OF *Aspergillus parasiticus* AND SPARGED WITH DIFFERENT MIXTURES OF OXYGEN AND CARBON DIOXIDE[a]

| | $O_2 : CO_2$ (9:1, v/v) | | | $O_2 : CO_2$ (1:1, v/v) | | | $O_2 : CO_2$ (1:9, v/v) | | |
	3 days	5 days	6 days	3 days	5 days	6 days	3 days	5 days	6 days
Aflatoxins[b]				(μg/500 ml)					
B_1	6	129	135	8	19	25	2	2	2
B_2	—[c]	13	16	2	2	3	—	—	1
G_1	12	103	126	8	30	34	2	3	3
G_2	—	—	11	—	3	4	—	—	—
Total	18	245	288	18	54	66	4	5	6
pH	5.8	3.9	3.5	5.8	5.4	4.2	6.2	5.8	5.10
Residual glucose (%)	3.85	3.28	1.70	3.90	3.70	3.30	4.75	4.05	3.50

[a]Flow rate of mixed gas = 0.5 ml gas/ml/min.
[b]Aflatoxins in broth.
[c]Not detected.

TABLE 3. AFLATOXIN FORMATION IN A LIQUID MEDIUM INOCULATED WITH SPORES OF *Aspergillus parasiticus* AND SPARGED WITH DIFFERENT MIXTURES OF OXYGEN AND NITROGEN[a]

| | $O_2 : N_2$ (9:1, v/v) | | | $O_2 : N_2$ (1:1, v/v) | | | $O_2 : N_2$ (1:9, v/v) | | |
	3 days	5 days	6 days	3 days	5 days	6 days	3 days	5 days	6 days
Aflatoxins[b]				(μg/500 ml)					
B_1	135	210	225	19	80	85	8	74	110
B_2	5	40	55	3	20	20	—[c]	11	20
G_1	175	280	285	45	67	67	6	35	52
G_2	20	30	35	4	15	7	—	8	7
Total	335	560	600	71	182	179	14	128	189
pH	5.8	4.8	4.2	5.8	4.4	4.3	6.1	4.5	4.4
Residual glucose (%)	3.66	3.56	3.40	3.60	3.06	3.20	3.94	3.88	3.38

[a]Flow rate of mixed gas = 0.5 ml gas/ml/min.
[b]Aflatoxins in broth
[c]Not detected.

gests that biosynthesis of the toxin occurred when available oxygen was somewhat limited. However, absence of O_2 or an excess of O_2 both inhibited aflatoxin formation.

DISCUSSION

Results of this study indicate that static incubation was best for maximal production of aflatoxin, although it was not optimal for mycelial growth. Increasing the rate of aeration reduced formation of toxin (Tables 1 and 4). Production of higher concentrations of toxin with reduced aeration also has been reported in the literature. Codner et al. (3) obtained 100-200 mg of aflatoxin per liter of medium in 250-ml flasks incubated on a rotary shaker, whereas the same strain of fungus failed to produce any aflatoxin in 3- and 20-liter stirred aerated fermenters. Davis et al. (4) indicated that one strain of A. flavus produced up to 63 mg of aflatoxin per 100 ml of YES medium when grown as a stationary culture in 1-liter flasks. However, this culture produced only 21 mg of toxin per 100 ml of medium in submerged culture with stirring and aeration (8). These results suggest that although O_2 is essential for growth and toxin formation, excessive amounts of O_2 reduce production of toxin.

Another factor which may cause the difference in production of aflatoxin between static and aerated cultures is the concentration of CO_2. It has been reported that low concentrations of CO_2 stimulate germination, growth, and metabolic activities of fungi, whereas high concentrations (above 20%) of CO_2 retard them (2, 7, 13, 18, 20). Barinova (2) studied the importance of CO_2 to the vital activity of fungi, and found that the gas stimulated growth of Aspergillus niger when grown in a CO_2-free medium. The effect of CO_2 tension on production of citric acid was studied by Vakil and Bhattacharyya (20) who indicated that withdrawal of the gas from the atmosphere surrounding growing Aspergillus niger cultures decreased the rate of citrate synthesis and hence the yield of citrate. Atmospheric CO_2 in small amounts accelerated growth of the mold and of citrate synthesis; the effect disappeared with an increasing concentration of CO_2.

According to Tabak and Cooke (19), low concentrations of CO_2 are involved in fungal metabolism leading to synthesis (fixation) of proteins, nucleic acids, and intermediates of the tricarboxylic acid cycle. Furthermore, CO_2 serves to catalyze the malonyl-coenzyme A system, which is involved in synthesis of fatty acids and of other natural products, including certain aromatic compounds. Hence, production of less aflatoxin in the aerated fermenter might have resulted because the concentration of CO_2 which was produced during fungal respiration was reduced, and thus it was not available for synthetic purposes.

Aflatoxin formation in an atmosphere of 90% O_2 plus 10% of N_2 was lower than that observed in an aerated (normal air) or static fermentation (Tables 1 and 3). The difference in toxin formation could have resulted from complete exclusion of CO_2 from the atmosphere during the fermentation. Similar results also were obtained when the mycelium was incubated quiescently and in an atmosphere of 90% O_2 and 10% N_2 (Table 4). These observations provide some evidence to indicate that low concentrations of CO_2 might have stimulated toxin formation.

When the environment contained more than 10% CO_2 (Tables 1 and 2), aflatoxin formation was markedly suppressed. Formation of aflatoxin was neg-

TABLE 4. AFLATOXIN FORMATION IN A LIQUID MEDIUM INOCULATED WITH MYCELIUM[a] OF Aspergillus parasiticus AND HELD UNDER DIFFERENT ATMOSPHERIC CONDITIONS[b] FOR 3 DAYS

	Mycelium (Inoculum)	Static culture	100% CO_2	100% N_2	O_2:N_2 (1:9, v/v)	O_2:N_2 (9:1, v/v)
Aflatoxins[c]			(μg/500 ml)			
B_1	21.5	1766	20.1	21.0	105	1442
B_2	11.6	464	8.5	9.5	31	289
G_1	6.0	1370	4.5	5.0	69	977
G_2	2.3	179	2.0	2.5	9	134
Total	41.4	3779	35.1	38.0	204	2842
Mycelium wt.[d] (g)	2.5	6.2	2.5	2.5	4.6	12.4
Aflatoxin produced μg/g mycelium	17.0	610.0	14.0	15.0	44.0	229.0
pH		3.9	5.4	5.2	6.0	4.8
Glucose residue (%)		1.6	3.2	3.2	3.2	2.4

[a]3-Day old mycelium was washed before inoculation; 2.5 g (wet weight) used for each experiment in the fermenter.
[b]The conditions were created by passing pure or mixed gas continuously through the fermenter except for the static culture, flow rate = 0.5 ml gas/ml/min.
[c]Toxins in mycelium and broth.
[d]Wet weight, mycelium was prepared by removing water through vacuum filtration.

ligible when the atmosphere was composed of 90% CO_2 and 10% O_2, and complete inhibition of aflatoxin synthesis occurred when the mycelium was incubated in an atmosphere of 100% CO_2 (Table 4). Inhibition by CO_2 of aflatoxin formation on peanuts was previously demonstrated by Landers et al. (9). They also showed that toxin formation was reduced by 70% when the CO_2 concentration was increased from 0.03% (air) to 20%. Toxin formation continued to be suppressed by higher concentrations of CO_2 (40-80%), and no aflatoxin was formed in an atmosphere of 100% CO_2.

The effect of mixtures of O_2 and N_2 on aflatoxin formation was similar to that observed with mixtures of O_2 and CO_2. However, there was less inhibition of toxin production by N_2 than by CO_2 (Tables 2 and 3). Incubation of the mycelium in an atmosphere of 100% N_2 completely suppressed aflatoxin formation. Introduction of O_2 into an atmosphere of N_2 caused resumption of toxin formation (Table 4). Results of this study show that the atmospheric condition favoring maximal growth of molds does not favor formation of the greatest amount of aflatoxin. Production of less toxin in an aerated culture suggests that O_2 and CO_2 may be involved in regulating aflatoxin formation. Further study is needed to completely explain these relationships. Furthermore, these results indicate that the optimal condition for aflatoxin production is quiescent incubation, and that suppression of toxin formation can be achieved by introducing high concentrations of CO_2 or N_2 into the environment. This information may be useful when packaging techniques are selected for use with foods that are likely to support mold growth and hence become contaminated with mycotoxins.

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ENTEROVIRUS PERSISTENCE IN SAUSAGE AND GROUND BEEF

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Abstract

Persistence of coxsackievirus type A9 suspended in ground beef was found not to be sufficiently affected by extensive bacterial growth, during periods of up to 8 days at 23 or 4 C, to afford any notable degree of protection to the consumer. Longer storage times resulted in marked virus loss. After 2 weeks at both temperatures >90% of the input virus was no longer infective. In the preparation of Thuringer sausage, approximately 85% of the input virus was lost during a 24-hr fermentation at 30 C. Subsequent heating of the prepared sausage at 49 C resulted in a progressive loss of virus. However, after 6 hr at 49 C, an average of 1.1×10^3 infectious units of virus per gram of sausage remained of an initial 7.5×10^5 infectious units per gram.

Studies in this laboratory (1) have shown that some enteroviruses are rapidly inactivated when exposed directly to growing cultures of proteolytic bacteria. However, the effect of microbial growth on survival of enteroviruses in foods has been studied only by indirect means. Lynt (10) tested the survival of several enterovirus types in a variety of foods, and concluded that decomposition which took place in foods stored at room temperature had no effect on virus survival. Only two of the food samples he tested showed marked bacterial growth during the storage time, pizza at 2.6×10^6 colonies per sample, and breaded shrimp, 2.9×10^6 colonies per sample (sample size not given). Kalitina (7, 8) experimentally contaminated autoclaved and non-autoclaved samples of mince meat and of cottage cheese with various enteroviruses, and found no significant difference in the persistence of virus in the paired samples. When survival times of enteroviruses in sterilized milk and in sour milk products were compared (9), the temperature of storage and the fermentation process did not substantially influence the dynamics of virus inactivation. The effect of suppressing bacterial growth in enterovirus-contaminated spaghetti by use of tetracycline was reported by Cliver et al. (3). The apparent decrease in putrefaction in the food sample containing the antibiotic did not enhance virus persistence.

Because of the lack of information on the effect that bacterial growth has on persistence of viruses in foods, we investigated the survival of a model enterovirus in two types of ground meat products: Thuringer

sausage, which undergoes a defined, non-proteolytic fermentation during its preparation, and market ground beef, which is often contaminated with both proteolytic and non-proteolytic bacteria. Although neither sausage nor ground beef has been directly implicated in outbreaks of enterovirus caused disease, enteroviruses have been isolated from market samples of ground beef (12). Coxsackievirus type A9 (CA 9) was selected as the model virus because it was more protease-sensitive than any of the other enteroviruses that we had tested.

Materials and Methods

Virus and tissue cultures

Coxsackievirus type A9 (CA9), strain Bozek, was obtained from the American Type Culture Collection. Tissue cultures used to propagate and titrate this virus were primary Rhesus (*Macaca mulatta*) monkey kidney (PMK) monolayers. The procedures to prepare these cultures and their use in virus titration in our laboratory have been described (2).

Sausage preparation and experimental contamination

Batter to prepare Thuringer sausage was obtained from Oscar Mayer and Co., Madison, Wis. The culture it contained was a *Lactobacillus* species. For determining virus survival, CA9 (10 ml of a stock virus suspension that had been extracted with ether and dialyzed overnight against 1 liter of distilled water) was added to 50 lb (22.7 kg) of sausage batter and mixed in by hand kneading. The batter was then stuffed into sausage casing, so that 10 or more sausages were obtained weighing about 3-4 lb (1.4-1.8 kg) each. After a 24-hr fermentation at 30 C, the sausage was heated at 49 C for periods up to 6 hr. The details of the apparatus and procedures used for the preparation of this sausage have been described (4).

Determinations of *Lactobacillus* concentration were made by surface inoculation of 0.1 ml quantities on APT agar (Difco). Virus content was determined by blending 11 g of sausage in 99 ml phosphate-buffered saline (PBS) in a Waring blender for 1 min. The homogenate was centrifuged at $2000 \times g$ (max) for 10 min. Five milliliters of supernatant fluid was added to an equal volume of cold diethyl ether and mixed for 2 min on a Vortex mixer. After refrigeration for 30 min, samples were centrifuged 20 min at $2000 \times g$ (max). The aqueous layer was diluted in PBS plus 2% agamma calf serum for virus titration in PMK tissue cultures.

Experimental contamination of ground beef

For these trials, 15 ml of stock CA9 diluted 10^{-1} in PBS were added to 600 g of ground beef (chuck) and kneaded in by hand. Four 150-g patties were made; these were stored at room or refrigerator temperature for 2 weeks. Eleven-grams samples were taken at the initial time and at intervals for 2 weeks, and treated in the same manner as described for sausage above. Plate count agar (Difco) was used for

bacterial enumeration; acidified potato-dextrose agar (Difco) was used for mold and yeast count determination.

RESULTS

Persistence of CA9 during sausage production

The results presented in Table 1 show that during the fermentation process at 30 C, approximately 85% of the input virus was lost. Heating the prepared sausage for 1, 3, and 6 hr at 49 C caused a progressive loss of virus. However, even after 6 hr of heating, an average of 1.1×10^3 plaque-forming units (PFU) of virus per g of sausage remained in the samples tested. The pH of the sausage before fermentation was 6.0; after fermentation, 4.8. The initial *Lactobacillus* concentration was 4.1×10^6 colonies/g. After fermentation, values for the four samples tested ranged from 7.0×10^8 to 1.5×10^9 colonies/g.

Persistence of CA9 in ground beef

Ground beef samples were stored either at room (23 C) or refrigerator temperature (4 C). At both temperatures, bacterial growth was rapid and reached high numbers per gram (Table 2). By 8 days, the total plate count was approximately the same at both temperatures for all samples. Of 5 different bacterial types, picked on the basis of colonial morphology, 3 from the samples that had been stored at room temperature were proteolytic. The concentration of molds and yeasts found in 4 days' incubation at both temperatures was so low ($< 10^3$ colonies per gram) that further counts were omitted.

At 4 C, 2 weeks were necessary before significant virus loss was noted. At 23 C, some loss occurred by 8 days' incubation, but 2 weeks were also required for a high degree of virus inactivation to occur.

DISCUSSION

The persistence of virus in experimentally contaminated ground meats did not appear to be significantly affected by the presence of bacteria, at least for the relatively short periods of storage time used here. In ground beef, CA9 was not rapidly lost, even though proteolytic bacteria were present in high numbers. By the time loss of virus became rapid enough to begin to afford some protection to the consumer (after 8 days at room temperature), the meat could hardly have been considered edible; it had undergone extensive putrefaction. The virus losses observed between 8 and 14 days may have been proteolytic. In the preparation of sausage, CA9 was able to withstand both the bacterial fermentation process and subsequent heat treatment. From these studies, it appears that enteroviruses are quite stable in ground meat products, which are among the most likely foods to become contaminated with virus through human handling.

A very significant observation during these studies was the relatively uniform distribution of virus found in each of the samples, both in ground beef and in

TABLE 1. PERSISTENCE OF CA9 IN SAUSAGE PRODUCTION

Time	Temp (°C)	Sample No.	Virus concentration (PFU/g)	Mean PFU/g	% PFU remaining
Initial time	—	1	7.7×10^5		
		2	7.8×10^5		
		3	7.2×10^5	7.5×10^5	—
		4	7.4×10^5		
24 hr fermentation	30	1	8.0×10^4		
		2	7.0×10^4	1.2×10^5	16.0
		3	1.7×10^5		
		4	1.4×10^5		
+ 1 hr	49	1	3.1×10^4		
		2	5.0×10^4	3.2×10^4	4.3
		3	2.1×10^4		
		4	2.5×10^4		
+ 3 hr	49	1	1.6×10^4		
		2	1.3×10^4	1.5×10^4	2.0
		3	1.7×10^4		
		4	1.6×10^4		
+ 6 hr	49	1	1.1×10^3		
		2	1.1×10^3	1.1×10^3	0.1
		3	9.0×10^2		
		4	1.1×10^3		

TABLE 2. PERSISTENCE OF CA9 IN GROUND BEEF

Storage temp (°C)	Sample replicate	Storage time (days)	Bacterial colonies/g	Virus PFU/g	% PFU remaining
4	1	0	7.0×10^5	9.3×10^4	—
		2	5.6×10^8	6.1×10^4	66
		4	3.8×10^8	8.4×10^4	90
		6	2.0×10^9	7.6×10^4	82
		8	6.0×10^9	7.8×10^4	84
		14	9.5×10^8	1.9×10^2	0.2
	2	0	6.5×10^5	1.1×10^5	—
		2	5.7×10^8	5.2×10^4	47
		4	9.0×10^8	9.8×10^4	89
		6	5.1×10^9	1.0×10^5	91
		8	4.8×10^9	9.3×10^4	85
		14	7.1×10^8	$<1.0 \times 10^2$	<0.1
23[a]	1	0	7.0×10^5	9.3×10^4	—
		2	2.2×10^9	8.6×10^4	92
		4	5.9×10^9	8.8×10^4	95
		6	3.9×10^9	2.6×10^4	28
		8	2.8×10^9	3.0×10^4	32
		14	7.2×10^8	1.1×10^1	0.01
	2	0	6.5×10^5	6.2×10^4	—
		2	5.8×10^7	6.2×10^4	100
		4	6.8×10^9	5.8×10^4	94
		6	6.1×10^9	1.8×10^4	29
		8	4.6×10^9	1.3×10^4	21
		14	1.1×10^9	$<1.0 \times 10^2$	<0.2

[a] Room temperature

sausage. This suggests that contamination of foods under natural conditions would result in a similar pattern of virus distribution. Thus, a random sample taken from a suspected food should contain detectable virus, provided that the level of contamination was sufficiently high. From the data reviewed by Grabow (5), the quantity of poliovirus excreted in feces during the carrier period averages 10^4 infectious units per gram. Thus, if 0.1 g was accidentally introduced into ground beef during the preparation of 100 beef patties (100 g each), the resulting concentration of 0.1 infectious unit per gram would be just below the sensitivity level for detection, based on current methodology (6). The total number of infectious units per 100-g patty (10) could, however, be sufficient to cause infection (11). At peak levels of enterovirus excretion, as many as 10^6 infectious units per gram of feces are present. Applied to the example given above, this quantity of virus would readily be detected, as well as being more than a sufficient quantity to initiate infection.

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FOOD PROTECTION DURING DISTRIBUTION[1]

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ABSTRACT

In-plant inspectors in federally-inspected meat plants are satisfactorily inspecting trucks that distribute meat and meat products In other food plants, inspections are more random and could be improved. Some truckers claim bias and lack of consistency in the acceptable degree of cleanliness. Refrigerated trucks on the road today are better than they have ever been before, and there are more of them. However, not all trucks meet the minimum standards of temperature protection and cleanliness. Food sanitarians can do a better job of checking trucks for their ability to safely transport perishable cargo, which would result in less claims damage and loss to both carrier and shipper.

As far as I can determine, sanitarians are not inspecting trucks. Outside of the USDA meat inspectors in plants shipping interstate, practically no one inspects trucks. Quality control inspectors do not inspect the trucks. Sanitarians do not inspect trucks. No one representing the shipper's interest sees the inside of a refrigerated truck, other than the dock hands and foreman.

This is what the truck lines tell us, and we asked quite a few of them. Many of these trucks haul more meat than anything else, so they do get inspected quite frequently at meat plants. State and city inspectors also get into the act on meat shipments.

But I still see meat being shipped in open trucks, pickups, compact vans, station wagons, trunks of automobiles, etc. And what about the foods other than meat—dairy foods, produce s u c h as lettuce, frozen foods, fish and other seafoods, tree fruits, and berries? I don't say that these foods need to be as closely regulated as meat and poultry now are, but from a quality control standpoint within a company, vehicles that transport and distribute foods could be more closely inspected at the loading dock to prevent food spoilage and eventual damage claims.

NEED TO CHECK TRANSPORTERS

There is a real need for food sanitarians or quality control people to check on their food transporters, both for cleanliness and for proper temperature control. Some outstanding examples might point up why.

Take New York, for example. I have always regarded New York City as having the most stringent food manufacturing codes. And it may be true. Everything is regulated, controlled, and prohibited in New York City. But that's as far as it goes. Nothing is enforced, at least as far as refrigerated trucks are concerned.

New York State may have the poorest refrigerated trucks in the nation. I remember visiting one of the larger refrigerated carriers in the state, and photographing his truck loading operation. The truck bodies were ancient, and they were in a terrible state of repair. When the last carton was crammed into the truck, the door was swung around, almost closed. Dock hands did not even attempt to close it all the way, for they had been unable to get it closed for some weeks. Instead, a wire was hanging on the door locking bar so they could wire the door in an almost-closed position, with a 2 or 3-inches gap left open. Sure, the refrigeration unit was turned on, and it would do some good refrigerating the cartons at the front of the truck. But the whole effect would be like driving in humid Houston with the windows down and the air conditioner on in our 100 F, 100% humidity weather.

While New York State may have the poorest refrigerated trucks in the nation, it is at least better than New York City. In the city, they don't even bother with a refrigerated truck. Instead, they use open pickups and light compacts of the Econoline variety to transport their meat. If you would visit the alleys behind many of those fancy restaurants, you would see their meat and perishable foods being delivered in dry freight vans, pickups, and compact vans. Some go as far as using an insulated van. But it probably does not have a refrigeration unit. Just an insulated van.

Veteran truck watchers, the men who spend more time looking at trucks than at girls, just don't see refrigerated trucks on New York City streets. I have been puzzled over this for many years, and several years ago I did some investigating to find out why. I found that there are regulations. City health department inspectors could reject incoming milk deliveries at a retail store if the temperature were over 50 F, and there are comparable meat handling regulations. Refrigeration is generally not required, but temperature control of the product is required, although hard to enforce.

Generally speaking, the enforcement divisions I talked to just didn't care or didn't know enough or

didn't have enough inspectors to do a job. The feeling was that the sun never shines in New York anyway, so there is no need to protect foods from heat damage during shipment.

I don't mean to pick on New York City. It is just the most outstanding example, being the largest city with probably the weakest enforcement. But the same thing happens in cities throughout the United States. I know that it happens in my city, Houston. A driver in Minneapolis, for example, told me he didn't care whether the refrigeration unit on his milk delivery truck worked or not. All he wanted was a unit to make some noise like it was running while he was unloading at some of the more quality-conscious supermarkets.

The noise of the unit has the opposite effect on some drivers. I inquired of a Mexican refrigerated carrier why he restricted his loads to frozen strawberries. He explained quite candidly that he did not have quite as much control over drivers in his country as we do here in the States. The driver is out there driving all by himself on lonely roads throughout the night. Noise of the refrigeration unit behind the cab gets to bothering him. So he turns off the refrigeration unit engine to get rid of the noise. For most of the 600 miles between the strawberry-producing regions around Irapuato to the border at Laredo, the truck is traveling without refrigeration other than that contained in the load itself. Frozen stawberries can stand that kind of abuse without it being detected, but fresh strawberries can't. Therefore, the frozen load is safer from a damage claim standpoint.

DECISIONS ARE A FACTOR IN COMPETITION

I think that you, as inspectors, have to appreciate that your decisions may be a competitive factor in the trucking of perishables. For example, right now in New York City, the reason we don't see many refrigerated trucks on the streets is that, competitively, most firms feel they can't afford the extra expense of insulated trucks and refrigeration units. Their competitors are not required to carry this extra expense, so why should they? Professional truck people tell us they can't sell, rent, or lease a decent refrigerated truck in New York City. The buyers don't want them. They're too expensive.

In cities where there is a fair amount of enforcement of food handling codes, the industry does some self-policing to this extent: A carrier who is doing a good job with clean equipment at the right temperature may be undercut in rates by another carrier with poor equipment or very little temperature control. The carrier with good equipment is going to encourage the Meat Inspection Division or local inspectors to check the carrier with poor equipment and so try to put him out of business—or at least force him to acquire better equipment and face the same costs. So the important thing in these regulations, aside from the primary purpose of protecting the wholesome quality of the food, is to make the regulations uniform and apply equally to everyone. This means enforcement should apply to the small truck operator as well as to the large one.

Inspectors also figure in the economic competitiveness of shipper versus carrier versus receiver. Not all truckers feel that inspectors have been fair in their judgments. Specifically, they state that it is almost impossible to get a written report from the receiving inspector telling why merchandise is rejected. And they say inspectors are not consistent—the same truck that was OK for loading last week will be rejected this week. They claim that inspectors tend to favor either the shipper or the receiver. When a load is rejected by the receiver, carriers question whether the real reason might not be that the market is down, or that the receiver may want to file a claim to help pay for the freight. For this reason, they have had to rely on independent inspectors to get a fair and unbiased report.

Claims are just another cost of doing business, and carriers know that if they are to be profitable, they must closely scrutinize the claims ratio. The better operated companies do this by using good equipment and keeping it well maintained so that it doesn't break down on the road. Even with good, reliable drivers who check the cargo temperature every few hours, though, they will still face a claim at destination for reasons beyond the control of the carrier. These reasons may range from too-warm cargo loaded at the shipper's dock to actual acts of God.

That is the reason for a little perishable claims manual entitled "Perishable Claims: The Problem and the Cure." It was written by a refrigerated carrier executive who had the benefit of many carriers' experience. It discusses the legal aspects of claims, citing court cases and prior decisions that affect claims payment. It also discusses how to prevent claims in the first place by proper cleaning of equipment, proper loading and unloading, and care of the perishable cargo while in transit. It includes frozen food handling codes adopted by industry as well as proper temperatures for several foods. I suggest that anyone involved with inspecting incoming or outgoing shipments might better understand the entire picture, including the economic benefits to his company, by having this 117-page book as a reference. It is published by the Common Carrier Conference-Irregular Route of the American Trucking

Associations, 1616 P Street NW, Washington, D. C. 20036.

For example, one of the useful items in this book is a two-page discussion of sour round in fresh meat. Many a carrier has paid claims for loss of quality because of "sour round, bone sour, or bone taint." However, the authority cited here shows how sour round can be caused by an excited animal with an increased level of propionate in peripheral blood and the carcass is not rapidly chilled after slaughter. In other words, the sour round can be caused at the packing plant rather than by any temperature conditions in transit. In the same manner, dark-cutting beef may be traced to ante-mortem stress. The author suggests that carriers consult a biological testing laboratory before p a y i n g claims on sour round. If the condition is caused by fatty acids, the carrier has no responsibility for the condition since it was set up before he took delivery on the meat.

CONCERNS OF INSPECTORS

Inspectors of transportation equipment, should be primarily concerned with the ability of the trailer or truck to get the perishable cargo to the destination without physical damage, bacterial damage, odor damage, and heat damage (or freeze damage). The best temperature for hauling much of the fresh produce like lettuce is as close to 32 F as possible without freezing. Several degrees lower and the lettuce will be ruined—carriers who get caught with their temperatures down become long-distance garbage haulers.

In inspecting equipment, realize that the manufacturers of trailers and truck bodies can easily determine the precise heat loss of their vehicles. Trailers have been tested accurately for heat loss for at least 12 years now, at the Budd Laboratories in Philadelphia, and at a similar facility at Miner Enterprises in Chicago. The only problem with these tests is that they are expensive—costing about $1,700 per test plus transportation costs to either of these cities. Now a new testing procedure developed by the Truck Trailer Manufacturers Association permits similar testing right in the trailer manufacturing plant or in the fleet garage. All that is required is an insulated test room big enough to test the trailer or truck body and about $3,000 worth of test equipment—mainly temperature recorders and heaters. The air leakage test which is done at the same time requires about $300 worth of test equipment.

Results of the test are stated as air leakage in terms of cubic feet per minute, and heat leakage in terms of BTU's per hour per °F difference between the ambient and the inside box temperature. A common standard is to consider the most extreme case —that is, of hauling frozen foods at 0 F on a 100-F summer day or a temperature difference of 100°. A well-insulated 40-ft. trailer as constructed today should test out at approximately 8,000 BTU's at 100° temperature difference, or 80 BTU's per degree temperature difference, or less.

As for air leakage, the USDA method that is performed at the Budd Laboratories in Philadelphia or the Miner Laboratories in Chicago tests air leakage of the van when it is pressurized to 0.1 inch water pressure, while the new TTMA in-plant test uses a pressure five times higher, at a 0.5-inch water column pressure. Therefore, the two tests aren't directly comparable. A test trailer that will leak 2 ft³/min pressure. A trailer leaking 4 ft³/min at 0.1 inch will at 0.1 inch pressure will leak 7 ft³ min at a 0.5 inch pressure. A trailer leaking 4 ft³ min at 0.1 inch will leak 11 ft³/min at 0.5 inch. And a trailer leaking 5 ft³/min at 0.1 inch will leak almost 15 ft³/min at 0.5 inch. These are actual test values taken from three different trailers.

Some new trailers will carry these ratings on a plaque or plate that can be checked. The values are determined when the trailer is new. The heat gain through the walls will not change much during the life of a trailer, but the air leakage rates can change drastically when door seals are damaged or worn. Of course, the air leakage rate affects the heat leakage, since the incoming warm air must be cooled.

These rating figures are especially meaningful now that we also have a BTU rating figure for the refrigeration units. The manufacturers of refrigeration units have agreed on a standard method of rating the cooling capacity at two temperatures, 35 and 0 F. The largest units made by the major manufacturers generally have a rating of approximately 18,000 to 20,000 BTU's per hour at 0 F in a 100 F ambient.

You can see that the largest refrigeration units generally have a cooling capacity about double that required to make up for the heat loss in the trailer when hauling frozen foods on a hot summer day, and about four times that required to haul fresh meat or produce. But that extra reserve capacity is not to be considered as useful for cooling the cargo. It is designed only to cool the air temperature inside the trailer after loading, and to provide a reserve in the event of equipment wear. It will also cool the trailer after door openings for drop shipments. But it is not possible for this doubled refrigeration capacity to keep the cargo at 0 F when making 15

or 20 LTL deliveries in a day.

I state this because shippers sometimes expect the carrier to cool their warm meat, or because frozen food shipments are questioned when they arrive at a few degrees above 0 F because the truck had to make a dozen door openings at previous stops. A carrier is only expected to deliver cargo at the same temperature at which it is loaded. He cannot be expected to cool in transit. We know that many meat packers do get overcrowded in the cooler and will ship warm meat. For this reason, many carriers require their drivers to take product temperatures on the dock before shipment. If the shipper loads and seals the trailer, then the driver will have to reach through the rear vent door to insert a thermometer in the product. Many trailers also are equipped with recording thermometers to protect the carrier from damage claims.

Besides a well insulated van with tight fitting doors and a refrigeration unit large enough to maintain product temperature, the inspector should inspect for cleanliness of the interior considering the product to be hauled.

A CLEAN TRUCK

If the trailer is designed and built properly, it can be easily cleaned. This includes fiberglass-reinforced plastic walls and ceiling, stainless steel meat rails, and tightly welded floor. Whether using corrugated aluminum refrigerator flooring or steel or aluminum flat plate, the floor seams can be welded to prevent water, blood, and meat residue from seeping into the insulation. When floors or walls are of wood, trailers are not so easily cleaned, and these demand closer inspection for odors, meat drippings, maggots, and other unclean conditions.

Sometimes foods get shipped by common carriers who normally haul only dry freight. There have been cases reported where foods such as lettuce were shipped in trailers that still had spilled agricultural chemicals on the floors—chemicals such as parathion, chlorodane, or DDT. This is an exception, and represents problems involved in trying to get by with someone other than a food carrier.

Generally speaking, meat trailers are steam cleaned just before picking up the load, and often cleaned again at the other end of the line after delivery. In this connection, there is a new phenomenon that is now puzzling carriers in regard to cleaning trailers. A microbiologist at the University of Georgia proposed a theory, substantiated by experiences of carriers, that loss of bloom or meat discoloration can be caused by oxides in the detergents used to sanitize the walls of the trailer. Experiments prove

that oxides left on the walls produce abnormal amounts of oxygen under the blast of air from the refrigeration unit, which in turn causes the meat to lose its bloom. One carrier is successfully resisting a claim because the shipper insisted the trailer be cleaned at the shipper's washing facility, and loss of bloom resulted from the cleaning chemicals. More research is going on and perhaps we will have more complete answers later.

Another condition that can cause loss of bloom is changing the temperature setting of the refrigeration unit. If the unit has been set at, say 28 F, and the trailer temperature is stabilized at that temperature, then changing to 34 F will cause the unit to introduce heat to raise the air temperature to that level. The meat may then become discolored during this heat cycle.

These are fine points, but they represent the state of the art of refrigerated transport today. We have solved the major technology and cost hurdles, and are now perfecting the fine points.

SOME PROBLEMS AND FUTURE DEVELOPMENTS

Refrigerated transport has never been better than it is today. The equipment is available for handling cross-country or around the corner almost any size shipment from 5 to 40,000 lb. When I say the equipment is available, I mean that many carriers, both private and for-hire, are using it. But not all. There are still some who try to get by with sub-standard equipment or non-refrigerated trucks, and those are the ones we are trying to convert. You hold an economic arm lock on them when you turn down these non-refrigerated or substandard vehicles.

There are still problems to be solved. One has to do with LTL shipments. It is not always possible to get delivery of small shipments to out-of-the way places like Muleshoe, Texas, or Wheelwright, Kentucky. What's more, it is very difficult to maintain the air temperature inside the trailer when making a large number of drop shipments. But through the use of curtains, bulkheads, and large capacity units, the industry is doing a very acceptable job and improving all the time.

One of the newest developments is a trailer design that the Department of Agriculture has been working on for some years. This prototype van trailer was shown at Transpo 72 in Washington in June. It is unique in that it achieves much better air circulation throughout the van. Air distribution is the really critical point today—surrounding the load with a blanket of cool air, and for some products, pushing the air through the cargo. In the USDA van, this is achieved by increasing the air

pressure more than five times with bigger fans, and by blowing this high pressure air down the sidewalls so that it comes out at floor level before rising up and flowing back to the front.

The larger fans provide air at about 2.2 inches of static pressure at the unit, or 1½ inches by the time it is distributed at the bottom of the sidewall flues, compared to about 0.2 inches external static pressure in most refrigeration units in use today. The air flows down the length of the trailer in two ceiling ducts and is forced down the flues between the insulation and the interior liner. It comes out at floor level. The floor is laid crosswise instead of longitudinally so that the air can flow under the load and up through it to the return air duct also in the ceiling. This trailer is designed so that the loaders can't block air passages when they cram in those last few cartons. It also has ports for injection of cryogenic gases for pre-cooling or in case of emergency malfunction.

In discussing some of the problems with refrigerated transportation, I hope you don't get the mistaken impression that the industry is suffering today from any really serious difficulties. Far from it. The state of the art is very high today compared with a few years ago—like proceeding from the ice age to the mechanized age. In the past 10 years practically the entire fleet of trucks and trailers on the road has been converted from various soggy insulations to the present standard, which is foamed-in-place polyurethane insulation. This foam insulation has about twice the insulation efficiency of any previous material and it tends to seal the van against air leakage. It does not become waterlogged, settle, or freeze the way other insulations did in the past. This is probably the single most revolutionary development in refrigerated transport in recent years, and the fact that the industry converted to it almost from the beginning is evidence of the desire to provide the best possible food transport.

Generally speaking, you can obtain today the same conditions of sanitation, refrigeration, and product protection in transit as in plants. It just costs more to provide it on the road.

A Research Note

FUNGI IN FOODS

V. RESPONSE OF NATURAL POPULATIONS TO INCUBATION TEMPERATURES BETWEEN 12 AND 32 C[1]

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Food Science Department

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Abstract

The effect of incubation temperatures from 12 to 32 C on yeast and mold counts was determined using both antibiotic and acidified media to control bacterial growth. Highest counts were obtained on the antibiotic medium. Lowest counts occurred with both media at 32 C. Little difference in recovery was apparent in the range of 12 to 27 C, with the fungi responding in a similar manner in both media to shifts in incubation temperature.

Earlier reports from this laboratory *(3, 4)* have pointed out the advantages of an antibiotic bacterial inhibitor in place of acidification for enumeration of yeast and molds in food. Ease of preparation, maximal recovery of fungi, and inhibition of acid-tolerant bacteria are some of the more obvious benefits gained by this substitution.

Since existing recommendations *(1, 2)* for incubation temperatures are probably based on earlier studies using acidified media *(5)*, it was believed necessary to determine what effect, if any, temperature had on recovery of fungi grown on the antibiotic medium. This was believed especially important in that incubation temperature was observed to have a marked effect on recovery in an earlier study *(3)*. Therefore, the effect of incubation temperature on recovery of fungi from foods was determined over the range of 12 to 32 C using an antibiotic medium in parallel with a standard procedure.

Materials and Methods

Samples

All samples were obtained from retail stores in the Gainesville, Florida area. Ten samples were analyzed from each of four food groups, representing frozen seafoods, dairy (mostly cheese), fresh meats, and vegetables.

Preparation of samples

Fifty-gram samples were blended in 450 ml of buffered dilution water *(1)* for 2 min. Additional dilutions were prepared by transferring 11 ml into 99 ml dilution blanks as needed. Ten sets of duplicate plates for each dilution were prepared. Five were poured with Potato Dextrose Agar (PDA)

adjusted to pH 3.5 ± 0.1 with sterile 10% tartaric acid and five PDA (pH 7) to which 100 mg/l each of chloramphenicol and chlortetracycline HCl were added after sterilization. A dilution series of each medium was incubated at 12, 17, 22, 27, and 32 C. To ensure maximum outgrowth of organisms, plates at 27 and 32 C were incubated for 5 days, those at 17 and 22 C for 7 days, and those at 12 C for 10 days.

Results and Discussion

The most notable temperature effect observed in this study (Fig. 1) was the pronounced reduction in counts at 32 C as compared to those at the lower temperatures. With the acid medium in particular,
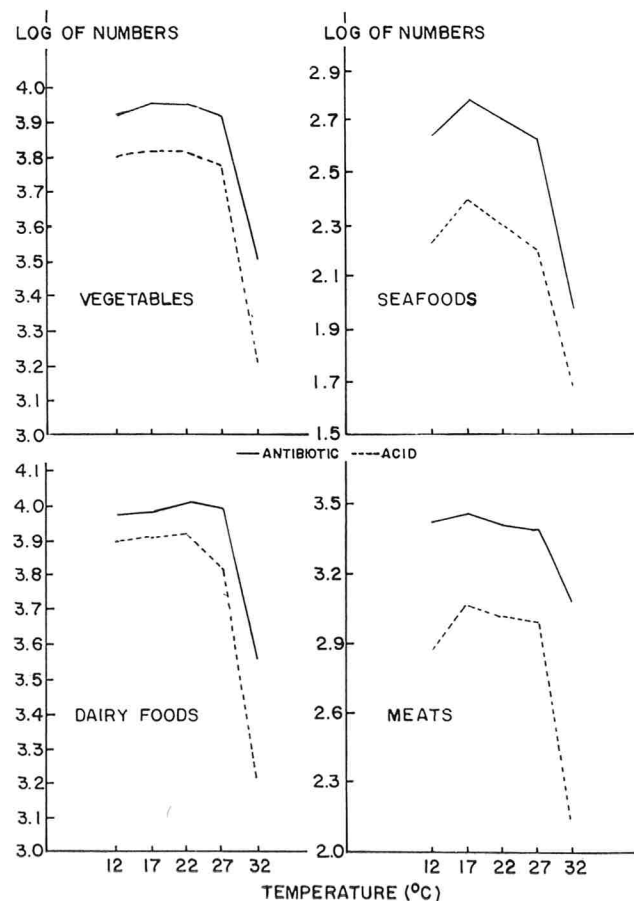
Figure 1. Effect of incubation temperature on recovery of fungi from foods.

a markedly lower count was observed at the highest temperature of incubation. From these results one immediately questions the value of a "total count" conducted at 32 C when the possibility exists that significant numbers of fungi are present.

Incubation at 12 C also resulted in somewhat lower recoveries as compared to the temperature which yielded the maximum count for each food group. However, as is found with so many food products, counts obtained at a lower temperature may be more informative as to past microbial activity and potential keeping quality than a count obtained at a higher temperature.

The consistency with which the antibiotic medium produced higher counts is apparent with all four groups of foods. Only rarely was a higher yeast and mold count obtained on the acidified medium. In regard to other temperatures, 17, 22, and 27 C, it appears that any incubation temperature within this range would produce about equal counts. It had been hoped that a shorter incubation period at a higher temperature would be possible with the antibiotic medium, however, this was not true. In fact, responses with the two media were remarkably similar. Although the stress of an acid environment is removed in the antibiotic medium, the flora, either indigenous or developed exhibits maximum growth over a relatively narrow range of temperature. Fortunately the more commonly used temperature of 22 C is within the range of maximum recovery.

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TWO AWARDS PRESENTED AT 1973 PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE
SIDNEY E. BARNARD
EXTENSION DAIRY SPECIALIST
THE PENNSYLVANIA STATE UNIVERSITY

Charles R. Brown, fieldman for Milk, Inc. received the 1973 Dairy Sanitarian Award. Dr. Howard K. Johnston, sanitarian, Pennsylvania Department of Agriculture received recognition for having served as chairman of the 1969 Interstate Milk Shippers Conference. Both men have been active in the Pennsylvania Dairy Sanitarians Association and their regional groups. Mr. Brown has aided many dairymen in their efforts to efficiently produce quality milk. Dr. Johnston has assisted both producers and processors in meeting the rigid dairy regulations.

More than 285 persons participated in the 31st Annual Pennsylvania Dairy Fieldmen's Conference. It was held at the Keller Conference Center on the University Park campus of Pennsylvania State University in State College, Pennsylvania June 11-13. Participants included industry farm inspectors, regulatory representatives, dairy processors, and allied industry personnel.

They were welcomed by James Beattie, who be-

Henry Wingert, Chairman, Awards Committee of Pennsylvania Dairy Sanitarians Association, is shown on the left presenting the 1973 Dairy Sanitarian Award to Charles R. Brown, Milk, Inc. on the right.

came Dean of the College of Agriculture on June 1. Many participants inspected the new milking facilities at the dairy center at Penn State. This includes a milking parlor, pipeline, precooler and 3,000 gallon storage tank. Mrs. Alexander and Mr. Fouse of the

Pennsylvania Department of Agriculture reported on current activities of their department and the discussion and action at the Interstate Milk Shippers Conference.

Mr. Mathis of Eastern Milk Producers Association reviewed the current milk supply situation. A quality control program for large cooperatives was outlined by Mr. Arledge of Dairymen, Inc. A panel outlined the trend toward automated testing for both quality and composition determinations. These hinge on the collection of an aseptic sample from each farm at every pickup.

Dr. Livak, Penn Dairies, Inc., discussed the Northeast Dairy Practices guidelines. These uniform recommendations have been approved by eleven northeastern states and cover housing, manure handling, milkhouses, sampling and related topics. Copies of the first seven guidelines are available from Richard March, Stocking Hall, Cornell University, Ithaca, New York 14850 for 25c each.

Other topics of current interest were welded stainless steel pipelines, legislation affecting rural Pennsylvania, treatment of farm water supplies, and potential contaminants in milk. A panel reviewed environmental pollution control as it relates to dairy farms. They covered waste management regulations, the Pennsylvania Clean Streams Act, and solid composting of animal wastes.

The dates for the 1974 Pennsylvania Dairy Fieldmen's Conference were set for June 10-12, 1974. Again it will be held at Penn State in State College, Pennsylvania starting Monday evening.

FRANK E. FISHER ELECTED PRESIDENT OF AFDOUS

Frank E. Fisher, Director, Bureau of Food & Drugs, Indiana State Board of Health, was elected President of the Association of Food and Drug Officials of the United States (AFDOUS) at their 77th Annual Conference in Rapid City, S.D. AFDOUS is comprised of state and local food and drug officials throughout the country and the professional staff of the U.S. Food & Drug Administration and the U. S. Department of Agriculture. Mr. Fisher has been a member of the Executive Board and has served on many committees of the Association. He was the recipient of the Harvey W. Wiley Award in 1970—the highest honor bestowed by the Association.

He is past president of the Central States Association of Food & Drug Officials and the Ohio Valley Conference of Food, Drug and Health Officials. He is a professional member of the Institute of Food Technologists, the International Association of Milk, Food and Environmental Sanitarians and the Indiana Public Health Association. He is a Diplomate of the American Intersociety for the Certification of Sanitarians.

Mr. Fisher has been employed by the Indiana State Board of Health since 1937 except for a period of service in the Army during World War II.

He is an instructor in the Department of Preventive Medicine at Indiana University School of Medicine and teaches Food Technology and Public Health Law.

DAIRY SCIENTIST RETIRES AT WASHINGTON STATE UNIVERSITY

A retirement dinner was recently held in Pullman for Dr. Louis J. Manus who has served Washington State University for 26 years. A native of the state of Washington, Dr. Manus finished his undergraduate work here under Dr. H. A. Bendixen, who was master of ceremonies at the retirement dinner. As an undergraduate, Louis was a member of Dr. Bendixen's dairy products judging team. Later as a faculty member Dr. Manus tutored dairy products judging teams for many years, bringing back numerous trophies in collegiate competition. His former students now hold responsible postitions where their quality control training has meant better dairy products for the consumer.

Dr. Manus received a Master's degree from West Virginia University in 1934. He was on the staff of that institution until 1941 when he started active military service as supply officer for the 99th Infantry Division, U.S. Army.

After the war Louis returned to Washington State where he completed work for a Ph. D. degree. Since then he has served on the faculty in Animal Sciences and later in the newly formed department of Food Science and Technology.

From 1964 to 1966 Dr. Manus served as Advisor in Dairy Science and Food Technology to West Pakistan Agricultural University. On the way home he attended the International Dairy Congress in Munich, Germany.

As a teacher Dr. Manus has been outstanding as shown by his selection this year to receive the RM Wade Foundation Award for "Excellence in Training". Throughout the years as a professor he has been a friend and inspiration to the students majoring in dairy and food processing..

For many years Washington State University conducted "Dairy Institutes" and short courses for dairy plant management and industry personnel. Dr. Manus served admirably as part of the team making these programs a success.

Dr. Manus has purchased a home in Marysville,

just north of Seattle, where he intends to enjoy his hobby of gardening.

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IRVING RE-ELECTED BEMA PRESIDENT

Frank M. Irving, Jr., President, Alto Corporation, York, Pennsylvania, was unanimously re-elected President of the Bakery Equipment Manufacturers Association at the Annual Convention held in Mexico, June 27-July 4, 1973.

Mr. Irving's first term in office as the Association's chief executive was outstanding. Many progressive measures were undertaken under his leadership and the Association has grown in size as well as in its scope of activities and services. He is also active in the American Society of Bakery Engineers, the American Retail Bakers Association, and is a Director of the American Institute of Baking. Mr. Irving is a member of the York Chamber of Commerce and is active in many civic affairs.

Other Officers unanimously re-elected were: First Vice President—Ervin V. Waack, Vice President, Baker Perkins Inc. Saginaw, Michigan; Second Vice President—W. E. Lanham, President, Lanham Machinery Company, Inc., Atlanta, Georgia; Raymond J. Walter continues to serve as the Association's Secretary-Treasurer and Counsel with Executive Offices being maintained in New York City.

Members of the Board elected or re-elected* were as follows: Director—Gilbert E. Good, National Sales Manager, Kwik Lok Corporation, Yakima, Washington; Director—*E. Thomas Oakes, President, Oakes Machine Corporation, Islip, New York; Director—*Quentin R. Russeth, Manager, Equipment Sales, Agri-Products Division, The Pillsbury Company, Minneapolis, Minnesota.

President Irving announced the appointment of the following members as BEMA's representatives to serve on the Board of the Baking Industry Sanitation Standards Committee: Ervin V. Waack, Chairman, Baker Perkins Inc., Saginaw, Michigan; Quentin R. Russeth, The Pillsbury Company, Minneapolis, Minnesota; W. E. Lanham, Lanham Machinery Co., Inc., Atlanta, Georgia; Bill Pulver (alt.), Velten & Pulver, Inc., Chicago Ridge, Illinois; Robert V. Loeb (alt.), E. J. Chubbuck Co., Inc., Englewood Cliffs, N. J.

_____

NEW PROCESS CONVERTS SOLID WASTE TO GAS

A new process that can turn solid waste into gas is being tried out at Orchard Park, near Buffalo, New York. The demonstration is being supported by the United States Environmental Protection Agency.

The facility can destroy limited quantities of such hard-to-dispose-of items as auto body parts, tires, tree stumps and limbs, plastics, refrigerators, clothes dryers and 55-gallon steel drums.

The process is being developed by Torrax Systems, Incorporated, of North Tonawanda, New York.

If the demonstration proves successful, it could have broad utility as a possible replacement for incinerators in large urban areas. Officials in such cities as Houston, Indianapolis, Jacksonville, Birmingham, New York, Washington, D. C., Chicago, and Boston are looking for ways to improve their solid waste management practices.

Present-day incinerators, according to EPA, have become increasingly expensive to equip and operate, because sophisticated air pollution control equipment is now required. In addition, these plants can reduce or destroy only about 50 percent, by volume, of the average municipal solid wastes. The remainder must be disposed of elsewhere. Land disposal is difficult near large cities because suitable land is scarce and expensive.

The process being tested near Buffalo is a combined combustion-pyrolytic system. Pyrolysis is the thermal reduction of materials in the near absence of oxygen. It works like this: Wastes are dumped into a large shaft furnace called a gasifier where they are subjected to blasts of air which have been heated to 2000 degrees Fahrenheit by natural gas.

The organic portion of the wastes then literally begins to decompose, and most of the waste volatilizes into gases. The nonvolatile portion of the organic wastes is burned to raise the temperature up to 3000 degrees Fahrenheit. At this temperature the nonburnable residues, which have settled to the bottom of the furnace, are liquefied into a molten slag.

The slag is water-quenched to form a fine granular material that is relatively chemically-inert. It may be made into building blocks and insulating fiber or may be used as a base material in highway construction. The gases from the gasifier can be burned to make steam for power generation. Appropriate experiments and testing will be made later.

Pollution control equipment is expected to be less costly in this system than that for standard incinerators, since pollution from the new system is expected to be one half of that produced in usual incineration methods.

Present costs of the pyrolytic-combustion process are from $12 to $14 per ton of waste processed, but the investigators are projecting $5 to $6 per ton in commercial operations. Normal incinerator costs run about $10 to $12 per ton. The sale of steam or slag, or both, as byproducts of the new process, could further reduce costs.

Capital costs, according to the Environmental Pro-

tection Agency, also will probably be lower.

For example, Chicago's new incinerator cost $28 million. The system under investigation, with the same total tonnage capacity, would cost between $18 to $20 million, according to the investigators. The system is much smaller than conventional incinerators of same capacity.

The EPA is supporting the demonstration with $1,421,195. The other participants—Erie County, the State of New York Pure Water Authority, the American Gas Association, A. E. Anderson Company, and Torrax Systems, Incorporated—are contributing approximately $500,000.

The EPA Project Engineer is Edward L. Higgins, 5555 Ridge Avenue, Cincinnati, Ohio 45268. Telephone: (513) 684-4338.

The Project Director is Charles C. Spencer, Erie County Department of Public Works, 95 Franklin Street, Buffalo, New York 14202. Telephone: (716) 846-8384.

NEW, PORTABLE BORESCOPE CARRIES OWN LIGHT SOURCE FOR FIELD INSPECTION

A new, portable industrial Borescope, with flexible fiber optics in a tough, armored cable, has its own battery-case light source for convenient field inspection and high resolution photography of otherwise inaccessible interior cavities. The interior of pipe, equipment, instruments or machinery having a 1/2 inch diameter or larger opening can be inspected for flaws, cracks or damage and manufactured products given final checkouts without costly, time-consuming disassembly.

Light travels along one fiber optic bundle to the areas of interest, providing cool, flat, shadowless lighting, ideal for precise, undistorted viewing and color photography. An image returns from the self-focusing DYONICS' lens along a second, coherent bundle to the eyepiece. This light source, without heat or wires, is safe for inspection aboard submarines or in tanks containing dangerous chemicals or explosive gases. A variable self-focusing lens system solves a major problem of small borescopes and provides constant focus from extreme closeups at one-eight inch (three millimeters) to a wide field of view, as the light-pipe is withdrawn to cover the desired area. With the 90° side-viewing mirror (.475 inches diameter), in place, sides of tubes or cylinders as small as 1/2 inch can be inspected up to the length of the fiberoptic light cable.

Complete standard unit consists of fiber optic light pipe in semi-flexible armored cable housing, three foot standard; lens tube with hard rubber eyepiece; battery case, straight viewing lens; 90° side-view mirror; and slim carrying case. An optional six-foot light cable is available. The new standard unit is, at less than five hundred dollars, believed to be the lowest-cost, high resolution borescope available with these features.

For more information, call or write John D. MacLean, Sales Coordinator, DYONICS, INC., 71 Pine Street, Woburn, Massachusetts 01801; Telephone: 617-935-5900.

EGG CHOLESTEROL NOT A SERIOUS HEALTH PROBLEM

Cholesterol in eggs is not a serious health problem for people. In fact, the real problem may be that people are eating fewer eggs because of incorrect information about cholesterol, says Art Maurer, University of Wisconsin-Extension food scientist.

Maurer notes that people incorrectly assume that by eating less cholesterol they will have less in their systems. Actually, a large amount of recent scientific research has shown this is not necessarily true. In fact, if we do not eat cholesterol in our foods, the body makes its own. The body makes about three-fourths of its cholesterol and one-fourth of its cholesterol comes from food eaten. And the body needs cholesterol—if we eat less, the body makes more.

Maurer says the amount of cholesterol in the blood depends on many things such as heredity, tension, type of work, exercise, smoking, and probably most of all, the amount of food eaten. Such things as total calories and obesity are very important.

The scientist notes that there are some people who may have cholesterol disorders, just as there are people who have diabetes. These people may need to watch their diet. But there is no research that clearly indicates that eating eggs will raise serum cholesterol levels in the average person.

Maurer reports that several new egg products have been developed for people who have real cholesterol disorders and for those people who eat fewer eggs because of doctors orders. These new products are low in fat and cholesterol.

SELF-INSPECTION PROGRAM FOR FOODSERVICE OPERATORS

The National Restaurant Association has just published "A Self-Inspection Program for Foodservice Operators on Sanitation and Safe Food Handling." This publication is actually a kit containing guidelines on how a foodservice operator can initiate a sanitation and safe food handling inspection program in his operation and also twenty-three (23) self-in-

spection checksheets covering functional activities and areas of a foodservice operation.

The Guidelines includes practical information on who within his organization should make inspections, what activities and facilities should be covered, how frequently inspections should be accomplished, and how the operator can organize a self-inspection program based on his use of those inspection checksheets which are applicable to his type of operation. The inspection checksheets cover personal safeness, food handling practices, facilities and equipment by functional areas and customer concerns.

The application of this program by a foodservice operator will result in his obtaining information which will be useful for his evaluation of the effectiveness of supervison and the adequacy of employee training, as well as bringing to light deficient practices and conditions requiring corrective action.

This comprehensive guidance publication can be obtained from the National Restaurant Association's Educational Materials Center, 1500 North Lake Shore Drive, Chicago, Illinois, 60610, at a single copy price of $2.50.

EMMETT BARKER TO KEY ASSOCIATION POST

Farm and Industrial Equipment Institute president John G. Staiger has announced the appointment of Emmett Barker, 35, as executive secretary and treasurer of the Institute, beginning August 1. Mr. Barker succeeds Douglas Hewitt who retired after 15 years with FIEI.

A 1960 graduate with a bachelor of science degree in agriculture from the University of Tennessee, Mr. Barker has had extensive experience in administration, organizational and program development work, public relations, and advertising and sales promotion.

He has been a consultant in the feed and frozen food industries; president and general manager of Agricultural Services Association, Bells, Tenn., an agricultural marketing organization; public relations director of the American Feed Manufacturers Association; and manager of advertising and sales promotion programs related to marketing livestock and poultry feeds with Security Mills, Inc., Knoxville, Tenn.

FIEI, based in Chicago, is a trade association whose member companies manufacture over 90 per cent of North American farm and industrial equipment—a $5.3 billion industry. Through its members and its communication and cooperation with related organizations and government agencies, FIEI activities encompass all aspects of the industry, including safety and comfort of the operators of its products, promotion

of the free world trade concept, engineering standardization for interchangeability of equipment, research, legislative, educational and many other matters. Along with its programs related to the entire industry, various FIEI councils are concerned with special product areas such as livestock and poultry equipment, farmstead equipment, crop drying, grain bins, milking machines, equipment for spraying and dusting for both farms and lawn and garden use, and the rapidly growing industrial equipment market. The latter group, the Industrial Equipment Manufacturers Council, has grown from 17 founding company members in 1959 to a current membership of 77.

Since its founding in 1893, FIEI has continually broadened its scope of activities in keeping with changing times and challenges, in the interests of efficiency and productivity of farmers and industrial equipment users.

UNIVERSITY OF FLORIDA SHORT COURSE

September 18-20, 1973—Short Course on "Automated Control Systems for the Food Industries" (production and quality assurance). University of Florda, Gainesville, Florida. Sponsored by the Florida Section Institute of Food Technologists and the Florida Cooperative Extension Service. Fee . . . $45.00. For further information write Dr. R. F. Matthews, Department of Food Science, University of Florida, Gainesville, Florida 32601.

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