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FOR 1979 ANNUAL MEETING

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Research Papers

Hazard Analysis and Control of Roast Beef Preparation in Foodservice Establishments
Frank L. Bryan* and Thomas W. McKinley .............. 4

Fate of Spoilage Microorganisms in Frozen and Chilled Concentrated Tomato Juice
B. J. Juven ........................................ 19

Quality Aspects of Commercial Frozen Minced Fish Blocks
J. J. Licciardello*, E. M. Ravesi, and M. G. Allsup 23

Effect of Inorganic Nutrients on Production of Steroid Glycoalkaloids by Phytophthora infestans Race 1.2.4
Melanie R. Maas*, Frederick J. Post, and D. K. Salunkhe ........................................... 27

Organochlorine Insecticides and Industrial Pollutants in the Milk Supply of Southern Ontario, Canada — 1977

Evaluation of Sauerkraut-Like Products from Direct-Acidification of Cabbage
D. Lonergan and R. C. Lindsay* ........................... 38

A Research Note: Proteolytic Activity in Ultra-Pasteurized, Aseptically Packaged Whipping Cream

Bacteriological Survey of Frozen Mexican-Style Foods Produced at Establishments Under Federal Inspection
Bernard F. Surkiewicz, Douglas F. Campbell, and Marshall E. Harris* ................................. 46

A Research Note: Behavior of Clostridium botulinum in Vacuum-Packed Fresh Celery
C. E. Johnson ........................................ 49

Inoculation of Citrie Acid-Fermenting Bacteria into Raw Milk in Farm Bulk Tanks
C. H. White* and S. G. Shilothi ........................................ 51

Chemical Destruction of Mycobacterium bovis in Milk
William D. Richards* and Charles O. Thoen .......................... 55

General Interest Papers

Tapeworms, Meat and Man: A Brief Review and Update of Cysticercosis Caused by Taenia Saginata and Taenia Solium
D. W. Hird and M. M. Pullen* ........................................ 58

Significance of Mycotoxins to Food Safety and Human Health
L. B. Bullerman ........................................ 65

A Field Topic: Effect of Disk Weight and Filtration Vacuum Level on Flow Time of Yellow Staining Milk Through Lintine Disks
D. K. O'Toole ........................................ 87

Asterisk indicates person to whom inquiries regarding paper should be addressed.

IAMFES Committee Reports ................................. 45,88
News and Events ........................................ 89
Coming Events ........................................... 90
Annual Meeting Registration Form ....................... 92
Awards Nomination Form ................................ 91
Index to Advertisers ..................................... 96
Membership Application Form ............................. 96

"Instruction to Contributors" can be obtained from the editor for the use of contributors of papers.

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Hazard Analysis and Control of Roast Beef Preparation in Foodservice Establishments

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(Received for publication January 3, 1978)

ABSTRACT

Roast beef preparation practices were examined in eight foodservice establishments for the likelihood of contamination and the possibilities of survival or growth during each step of the operations. Clostridium perfringens was isolated from raw beef, equipment, and cooked beef. Staphylococcus aureus was isolated from raw beef, equipment, workers' hands, and cooked beef. Salmonellae were isolated from neither meat nor equipment. Numerous opportunities were observed for contamination of cooked beef during operations in most of the establishments. No opportunities for multiplication of foodborne disease bacteria were observed during thawing of frozen beef. From recorded temperature data, it was calculated that vegetative foodborne pathogens could survive in 76% of the geometric centers and on 5% of the surfaces of beef during cooking. Survival of these organisms could occur in 36% of the geometric centers and on 11% of the surfaces of the cooked beef during post-oven temperature rise periods. These organisms could have survived in 25% of the geometric centers and on 33% of the surfaces of the cooked roasts during hot holding; they could have multiplied on 25% and 27%, respectively. During cooling, the potential for multiplication of vegetative cells of foodborne pathogens existed in 83% of the geometric centers and 79% of the surfaces of the roasts. During reheating, these organisms would have survived in 71% of the geometric centers and on 13% of the surfaces of the roasts. Recommendations are given for hot holding, cooling, and reheating so as to minimize microbiological problems.

Roast beef is popular for banquet meals, for entrees in cafeterias and table-service restaurants, and for sandwiches in fast-service (fast-food) restaurants. As the standard of living continues to rise, the demand for it increases. Roast beef also, however, is the most frequently reported vehicle for the foodborne disease outbreaks that have occurred in the United States in recent years (7).

Raw beef can be contaminated with foodborne pathogens before it enters a kitchen, or contamination can occur in the kitchen (2,4). Roasts that are implicated as vehicles in outbreaks of foodborne disease frequently have a history of having been served in a foodservice establishment and either of having been held at room temperature, of having been held in hot-storage devices, or of having been refrigerated in large masses for several hours, or any combination of these treatments (2,3). If prepared-and-stored or leftover roasts are involved as vehicles in outbreaks (and they often are), they have usually been inadequately reheated before serving.

The purpose of this study was to identify foodborne disease hazards in preparation of roast beef by examining a variety of methods of preparation that are in current use and to suggest some corrective actions that should be taken. This was accomplished by assessing opportunities for survival during cooking and reheating, post-cooking contamination, and multiplication during room-, refrigerated-, and hot-storage in several different types of foodservice operations.

MATERIALS AND METHODS

Hazard analyses were made of 11 roast beef operations in eight foodservice establishments during the period 1972 to 1977. Table 1 describes the type of service and methods of cooking and hot holding used in each of these operations. Roasts were prepared from either whole cuts of beef or from chunks of raw beef mixed with ground raw beef that was molded and packaged. The establishments were surveyed over a 3- to 4-day period so that at least two complete preparation cycles were evaluated. Selection was based upon establishments that had operations that were different from one another but yet representative of operations throughout the industry. Two of the establishments previously prepared foods that were responsible for foodborne disease outbreaks. Workers were asked to prepare roast beef in their routine way and were not given any special instruction. Modifications in routine practices were occasionally made by the investigators to evaluate a specific procedure that might be useful as a recommended method.

Sources and routes of contamination

Operations were observed for possible sources and routes of cross-contamination from raw beef to cooked products, for post-cooking contamination by workers or equipment, or for other circumstances which could have led to contamination. Raw and cooked beef, beef jus and its ingredients, and a few workers' hands were examined for the presence of Clostridium perfringens, Salmonella, and

1 Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Department of Health, Education and Welfare or the Georgia Department of Human Resources.
2 Center for Disease Control.
3 Georgia Department of Human Resources.
HAZARD ANALYSIS OF ROAST BEEF PREPARATION

Staphylococcus aureus during sequential steps of preparation in three establishments. Sampling for pathogens was done by simultaneously rubbing four cotton swabs randomly over surfaces of raw beef, cooked roasts, and surfaces of equipment that the beef touched during preparation. Each of two of the swabs were put into two separate tubes containing cooked meat dextrose broth; another was put into a tube of tetrathionate brilliant green broth (Difco); the last was put into a tube of trypticase soy (BBL) broth. Tubes were held at room temperature until sampled (within 24 h). Samples of dried ingredients, stock, and beef j us were aseptically put into sterile plastic bags. Stock and beef j us were held at 7 C or lower until cultured (within 24 h). Hands were sampled by pouring 50 ml of trypticase soy broth over workers’ hands while they wiped their hands with a 4-inch sterile gauze pad; the broth was collected in sterile pans and transferred with the gauze pad to a Whirl-Pak bag for transport to the laboratory. Separate roasts from those in which thermocouples were attached, but from the same production lot, were selected for microbiological sampling. Samples of both raw and cooked beef were collected.

One tube of cooked meat dextrose broth was heated to 80 C for 10 min, the other tube was not heated. Tubes of cooked meat broth were then incubated anaerobically at 37 C for 24 h. The incubated, cooked meat broth was inoculated into tubes of thioglycolate broth and incubated aerobically for 24 h. The incubated thioglycolate broth tubes that showed gas were streaked onto McClung-Toahe egg yolk (laboratory-prepared). Suspicious colonies were picked and confirmed or ruled out as C. perfringens by gram staining, inoculated into indole-nitrite broth (BBL), litmus milk (Difco), and motility medium (Difco), and streaked (colonies from blood agar plates) onto egg yolk agar. Ten grams of dried ingredients were added to 100 ml of 0.1% peptone water. Beef j us was shaken, and then 1 ml was added to each of two tubes of 10 ml of cooked meat dextrose broth. These samples were then examined as described for the swab samples.

Twenty milliliters of beef j us were added to 200 ml of tetrathionate broth containing brilliant green and turgitol. Tetrathionate broth tubes were incubated at 37 C overnight and then streaked to brilliant green agar plates. After 18 to 24 h of incubation at 37 C, suspicious colonies were picked to triple sugar iron agar slants. These slants were incubated overnight at 37 C. Suspect cultures were confirmed or rejected as Salmonella by urease testing and slide agglutination.

Trypticase soy broth tubes were incubated overnight at 37 C. The incubated broth was streaked to tellurite polymyxin egg yolk (Difco) agar and incubated overnight at 37 C. Colonies suspected of being S. aureus were picked for testing for coagulase production, using the tube method. Samples of beef j us were streaked directly onto tellurite polymyxin egg yolk plates and examined as were the swab samples.

Time-temperature evaluations

Type-T thermocouples were inserted into cuts of beef by pushing the sensing-end into the geometric center or other designated location of thawed meat. If the meat was frozen, a hole, the diameter of the thermocouple wire, was drilled to the geometric center. To evaluate surface temperatures, either soldered-end thermocouple sensors were inserted just under the skin or under a muscle sheath, or button-type thermocouples were pressed against the meat surface and secured by wrapping the lead with insulated wire and lacing the wire into the meat. Temperatures of cooking units, of hot-holding devices, and of refrigerators were evaluated with soldered-end thermocouples placed within a few inches of the meat being processed or stored. Thermocouple leads were attached to the terminals of a potentiometer, and temperatures were recorded on a chart. Whenever possible, thermocouples were left in place during subsequent phases of roast beef preparation. Bits and thermocouples were washed.

*Laboratory analyses were done by the Special Studies Laboratory, Division of Physical Health, Georgia Department of Human Resources, Atlanta, Ga.

1 Temperatures were recorded on chart 5270 (type T), 0 to 500 F range in an Electronik 16 Multipoint Recorder, Honeywell, Fort Washington, Pa.
dipped in alcohol, and flamed three times before inserting into roasts. Procedures of attachment and location followed those described by Bryan and Kilpatrick (4) and Bryan and McKinley (5).

RESULTS

Sources and routes of contamination

Raw beef, dry beef-jus mix, and workers' hands were identified as sources of contamination for products (Table 2). Samples of raw beef frequently contained C. perfringens and S. aureus. Dried beef-jus mix also contained C. perfringens. Workers' hands harbored S. aureus. Eight percent of swabs from equipment (knives used for boning raw meat and for slicing cooked roasts) yielded C. perfringens. Six percent of swabs of equipment (wooden table tops in the raw preparation area and knives used for boning and for slicing cooked beef) yielded S. aureus.

There were opportunities for contamination during some of the operations. These include workers' touching cooked surfaces of roasts with hands during carving, trimming, boning, wrapping, and unwrapping; touching roasts with hands during cooking to check doneness; touching roasts with hands during room-temperature storage to check whether cool enough to put in refrigerators; handling (after touching raw beef) salads, boiled shrimp, and other foods intended to be eaten without subsequent cooking; grinding cooked leftover roasts in grinders that had been used for raw meat without subsequent cleaning and disinfection; cutting cooked roasts and bell peppers on cutting boards on which raw roasts had been processed and which were not cleaned and disinfected before being used for the cooked meat; using the same scales to weigh raw meat for kabobs and to check moisture loss of cooked roasts; and using contaminated knives, slicers, cutting boards, and storage pans for cooked roasts.

Survival or growth potential

The bases for interpreting expected survival or growth, if the roast had been contaminated, are as follows: Data about cooking, hot holding, and reheating are interpreted according to the potential for destruction of large numbers (10^7) of vegetative foodborne bacteria. Destruction is calculated upon the following criteria: 1 sec at or above 73.9 C (165 F); 12 min or more at or above 65.6 C (150 F); and 83 min or more at or above 60 C (140 F) (I). Data given in tables about thawing, cooling, hot holding, and reheating include the temperature range from 21 to 46 C (70 to 115 F), which is an approximate range within which mesophilic bacteria multiply rather rapidly; from 29 to 46 C (85 to 115 F), which is a range that is closer to optimal temperatures for foodborne disease bacteria; from 15.6 to 50 C (60 to 122 F), which is an approximate range that C. perfringens could grow on cooked meat surfaces or in the interiors; and 7 to 60 C (45 to 140 F), which is the range specified in foodservice code requirements [cold-stored foods should be 7 C (140 F) or below, and hot-held foods should be 60 C (140 F) or above]. Data are interpreted according to the potential for growth of mesophilic or pathogenic foodborne disease bacteria that are characterized by a lag phase of at least 60 min and additional time for one or more generations at the 21 to 46 C (70 to 115 F) range. Differences of pH, oxidation-reduction potential, and water activity between internal regions and surfaces were not considered in making the evaluations.

Thawing

During thawing at room temperature or in refrigerators, temperatures of outside surfaces and internal regions of roasts did not rise high enough for long enough to permit multiplication of foodborne disease bacteria (Table 3, Fig. 1). No roast surface reached 21 C (70 F). One roast, which was put into a refrigerator to thaw, had not done so after 40 h.

Cooking

During cooking, beef surfaces reached sufficiently high temperatures for sufficiently long periods of time to kill vegetative foodborne disease bacteria, except for one occasion when a 1.6-pound roast was cooked for only 34 min (Table 4). Geometric centers of roasts, other than those barbecued, seldom reached time-temperature values lethal to vegetative foodborne disease bacteria.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clostridium perfringens</th>
<th>Salmonella</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number sampled</td>
<td>Number positive</td>
<td>Percent</td>
</tr>
<tr>
<td>Products and ingredients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw beef</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Cooked meat</td>
<td>19</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>jus</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef stock mix</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Caramel coloring</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Workers' hands</td>
<td>26</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Equipment</td>
<td>60</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

1 Equipment examined: top of wooden table in raw preparation area, before and after boning; knife before and after boning; cutting board on serving line; fork on serving line; grinder before and after grinding cooked meat; storage pan; thermometer; slicer for cooked meat, scale for cooked meat; waste pan for slicer.

Samples positive for C. perfringens were knife for cooked meat (1/7), knife after boning (1/1). Samples for S. aureus were top of wooden table before boning (1/1).
Typical situations of roasting beef are illustrated in Figures 2-6. On the basis of the criteria set, foodborne pathogens (if present) would have survived in the geometric center of 19 (76%), possibly in 23, of 26 cooked roasts and on the surfaces of 1 of 21 (5%) of cooked roasts.

**Holding at room temperature after cooking**

After removing roasts from the oven, surfaces cooled immediately, but for a period of time the temperature rose in the interior of the roasts at the same rate as during cooking (Table 5). This period varied in 4.8-lb. to 26-lb. roasts from 12 to 92 min and depended on the rate of temperature rise during cooking and the size of the roast. On the basis of the criteria set, foodborne pathogens (if present) would have survived in the geometric center of four (36%), possibly six, of 11 cooked roasts and on the surfaces of one of nine cooked roasts. During holding situations, when temperatures of cooked surfaces or internal portions were initially low, temperatures were in a range in which foodborne disease pathogens would have survived in the geometric center of four (36%), possibly six, of 11 cooked roasts and on the surfaces of one of nine cooked roasts. During holding situations, when temperatures of cooked surfaces or internal portions were initially low, temperatures were in a range in which foodborne disease pathogens could multiply. Examples of time-temperature conditions to which roasts were exposed during room-temperature-holding are illustrated in Figures 3, 5, and 6.

**Holding in hot-storage devices**

When roasts were taken out of ovens and immediately put into hot-holding devices, there was a post-oven temperature rise in the central region of the meat (Table 6). When roasts were put into hot-storage devices which had air temperatures of 67.8 C (154 F) or higher, beef surfaces and internal temperatures frequently reached temperatures sufficiently high and stayed there long enough to expect destruction of vegetative foodborne disease bacteria (Table 6; Fig. 7). During five (25%), possibly eight, of 20 evaluations of the geometric centers and four (27%), possibly six, of 15 evaluations of surfaces, roasts were held at temperatures in hot-storage devices for sufficient periods of time that, if roasts were contaminated, spore germination and considerable growth of foodborne disease bacteria would be expected (Table 6; Fig. 8 and 9).

**Cooling**

During cooling, 20 (83%), possibly 21, of 24 geometric centers and 11 (79%), possibly 12, of 14 surfaces of roasts (except slices of cooked meat and roasts weighing less than 1.25 lb.) were exposed to incubation temperatures long enough to have permitted growth of mesophilic bacteria, including C. perfringens, if they had been present (Table 7). Typical cooling curves are illustrated in Fig. 10 and 11. The period that temperatures were within 7.2 to 60 C (45 to 140 F) range frequently exceeded 4 h and was usually more than 8 h. Little difference was observed in the cooling rates of roasts wrapped in foil or plastic film (Fig. 10). When several roasts were stored in plastic pans covered with lids in a walk-in refrigerator, surfaces as well as internal portions of the roasts cooled very slowly (Fig. 11).

**Reheating**

During reheating chilled roasts, surfaces of 13 (87%) of 15 reached high enough temperatures for long enough to expect destruction of vegetative mesophilic bacteria (Table 8). On 10 (71%) of 14 occasions, however, the internal temperatures did not. (Typical heating curves are illustrated in Fig. 12-17). During reheating, temperatures of different surface sites differed, but usually they reached 73.9 C (165 F) or higher within an hour (Fig. 12). In one operation, temperature conditions during

![Figure 1. Temperatures of a surface location and approximate geometric center of a raw, 7.5-lb cut of beef while being thawed at room temperature.](image-url)

<table>
<thead>
<tr>
<th>Thawing procedure and temperature</th>
<th>Frozen weight (pounds)</th>
<th>Probe positions</th>
<th>Holding period (min)</th>
<th>Minutes from 32 to 44.9 F</th>
<th>Minutes from 45 F to final temperature</th>
<th>Final temperature F</th>
<th>Potential for growth of foodborne disease bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature (64 to 72 F)</td>
<td>8.75</td>
<td>S</td>
<td>1080</td>
<td>803</td>
<td>217</td>
<td>55</td>
<td>—</td>
</tr>
<tr>
<td>Room temperature (66 to 73 F)</td>
<td>7.5</td>
<td>GC</td>
<td>1080</td>
<td>215</td>
<td>75</td>
<td>50</td>
<td>—</td>
</tr>
<tr>
<td>Fabricated beef in refrigerator (31 to 46 F)</td>
<td>10</td>
<td>GC</td>
<td>660</td>
<td>230</td>
<td>430</td>
<td>66</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Fabricated from small chunks of beef and ground beef.
2 S = surface; GC = geometric center.
3 * indicates expected multiplication of foodborne disease bacteria; — indicates no expected growth.

<table>
<thead>
<tr>
<th>Thawing procedure and temperature</th>
<th>Frozen weight (pounds)</th>
<th>Probe positions</th>
<th>Holding period (min)</th>
<th>Minutes from 32 to 44.9 F</th>
<th>Minutes from 45 F to final temperature</th>
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<tr>
<td>Room temperature (64 to 72 F)</td>
<td>8.75</td>
<td>S</td>
<td>1080</td>
<td>803</td>
<td>217</td>
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<tr>
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<td>7.5</td>
<td>GC</td>
<td>1080</td>
<td>215</td>
<td>75</td>
<td>50</td>
<td>—</td>
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<td>660</td>
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<td>430</td>
<td>66</td>
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1 Fabricated from small chunks of beef and ground beef.
2 S = surface; GC = geometric center.
3 * indicates expected multiplication of foodborne disease bacteria; — indicates no expected growth.
<table>
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<th>Cooking procedures</th>
<th>Raw weight (pounds)</th>
<th>Probe position</th>
<th>Cooking period (min)</th>
<th>Cooking unit temperature (°F)</th>
<th>Minutes at 140 to 149.9°F (min)</th>
<th>Minutes at 150 to 164.9°F (min)</th>
<th>Minutes at and above 165°F (min)</th>
<th>Highest temperature (°F)</th>
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<td>0</td>
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<td>237</td>
<td>350 - 500</td>
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<td>0</td>
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<td>173</td>
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<td>5</td>
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<td>148</td>
<td>208</td>
<td>+</td>
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<tr>
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<td>430</td>
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<td>7</td>
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<td>S</td>
<td>217</td>
<td>245 - 340</td>
<td>4</td>
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<td>S</td>
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<td>250 - 360</td>
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<td>S</td>
<td>235</td>
<td>245 - 387</td>
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<td>272 - 302</td>
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<td>12</td>
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</table>
HAZARD ANALYSIS OF ROAST BEEF PREPARATION

Figure 2. Temperatures of surface and internal locations of a 50.75-lb. round of beef while being cooked in an oven.

Figure 3. Temperatures of surface and internal locations of a 26-lb. prime-rib of beef while being baked in rock salt.

Figure 4. Temperatures of a surface location and approximate geometric center of a 7-lb. cut of beef while being roasted and held in an oven-warmer.
reheating of chilled, 18- or 26-lb. roasts in a steam-pipe-heated warming cabinet were such that significant bacterial growth could have occurred (Fig. 14).

DISCUSSION

There are many opportunities for contamination of beef with foodborne pathogens before it arrives at foodservice establishments and within these establishments. Therefore, beef must be treated during each operation as if it were contaminated. Most contamination is on beef surfaces, and surfaces usually reach lethal temperatures (73.9 C; 165 F or higher) for vegetative foodborne disease bacteria during cooking, unless the dehydration during cooking lowers the water activity to a point that would provide protection to the contaminants. Internal contamination can occur, however, when proteolytic bacteria break down connective tissue between muscle fibers and penetrate the interior of the meat (9), when interior lymph nodes are infected, when the meat is boned or rolled, or when thermometers or needles (for adding tenderizer) are inserted. Destruction of microorganisms that are in the subsurface portions from whatever source cannot be assured with most contemporary cooking methods.

Because of the taste preferences of the American public, and because of industrial practices, beef is seldom cooked to internal temperatures that would be lethal to foodborne pathogens. This usually causes no risk of bacterial foodborne illness, however, if the beef is eaten immediately after cooking.

Additional heat treatment occurs during the period of post-oven temperature rise at room-temperature storage or in hot-storage devices. Oftentimes, destruction of vegetative bacteria that are not killed during cooking would occur during this period. The time-temperature effect of post-oven temperature rise should be taken into account when evaluating the effectiveness of cooking in killing microbial populations.

Hot-storage leads to either destruction of vegetative bacteria or germination of spores and growth of mesophilic or thermophilic bacteria, depending on initial temperature of roasts when they are put into hot-storage units and the temperature of the units. Destruction of vegetative foodborne disease bacteria usually would be expected in roasts which entered hot-storage units at temperatures above 62.8 C (145 F), but it also could be expected to occur in roasts at any initial temperature that are put in hot-storage units with air temperatures continuously at or above 68.3 C (155 F), in the coldest zone, if they remained in these units long enough. Often they do not during periods of peak sales. Post-oven temperature rise varied because of the rate of heating rise during cooking, the size of the roast, the end-point temperature, and the temperature of the unit in which roasts were placed after cooking. Growth of vegetative bacteria would be expected when roasts remain in hot-storage units in which the unit air temperature is less than 62.8 C (145 F), in the coldest zone, for long periods of time.

Critical concern should be given to the hot-storage of roasts, particularly if the storage period is for several hours. Temperatures of geometric centers of roasts should remain at or exceed 54.4 C (130 F) to assure safety. Bryan and Kilpatrick (4) suggested that a cleaned and disinfected thermometer be inserted into a roast of typical size and dimension to be representative of roasts cooked in the same oven compartment at the same time and stored in the same hot-storage unit at the same time.
TABLE 5. Time-temperature exposure of cooked beef while at room-temperature storage between cooking and storage in hot-holding devices.

<table>
<thead>
<tr>
<th>Product</th>
<th>Raw Weight (pounds)</th>
<th>Probe position</th>
<th>Holding period (min)</th>
<th>Initial temperature (F)</th>
<th>Highest temperature (F)</th>
<th>Final temperature (F)</th>
<th>Post-oven temperature rise period (min)</th>
<th>Post-oven temperature rise period (F)</th>
<th>Minutes at 140-169.9 F (F)</th>
<th>Minutes at 150-164.9 F (F)</th>
<th>Minutes at or above 165 (F)</th>
<th>Potential for survival of vegetative foodborne disease bacteria²</th>
<th>Potential for growth of foodborne disease bacteria³</th>
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¹ S = surface; GC = geometric center; NC = near center; 3/4, 1 1/2 inches in roast; M = equal distance between surface and geometric center; U = unknown weight, approximate weight in parentheses.

² + indicates expected survival of vegetative foodborne disease bacteria; - indicates expected destruction of large numbers of large numbers of vegetative foodborne disease bacteria.

³ + indicates expected multiplication; - indicates no expected multiplication.

² Roasts fabricated from small chunks of beef and ground beef.
<table>
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<th>Feature</th>
<th>Heat holding device temperature</th>
<th>Raw weight (pounds)</th>
<th>Probe position1</th>
<th>Holding period (min)</th>
<th>Initial temperature (°F)</th>
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<th>Final temperature (°F)</th>
<th>Post-oven time (min)</th>
<th>Minutes at 140°F (min)</th>
<th>Minutes at 160°F (min)</th>
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<td>GC</td>
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<td>GC</td>
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<td>146</td>
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1 S = surface; SJ = just under surface; GC = geometric center; U = not measured.
2 + indicates expected survival of vegetative foodborne disease bacteria; + indicates expected destruction of large numbers of vegetative foodborne disease bacteria.
3 Indicates expected multiplication of foodborne disease bacteria; ++ indicates expected multiplication reaching large numbers; +++ indicates expected multiplication reaching very large numbers (outbreak potential); - indicates no expected multiplication.
4 Fabricated from small chunks of beef and ground beef.

**TABLE 6: Time-temperature exposure of cooked beef roasts during storage in heat-holding devices immediately after cooking or after room temperature-holding after cooking.**
controls are often inadequate, hot-storage devices sometimes malfunction (6), power or steam failures do occur on occasion, hot-storage units are sometimes inadvertently or purposely turned off (4,5), thermophilic bacteria can grow during longer storage, and new untrained employees are not always aware of the hazards and need for adequate temperature controls. The chances for error are too great. Managers should discontinue any such practice in their operations, and health authorities should supervise to see that such practice is not used.

Large cuts of beef cool quite slowly. Germination of spores and growth of vegetative foodborne disease bacteria can occur if roasts are stored at room temperature, if several roasts are stored in the same container in refrigerators, if roasts are stored in deep containers in refrigerators, and even if roasts which are bulky are stored singly in refrigerators. Data in this study (Table 7; Fig. 10 and 11) show that internal portions of roasts frequently had temperatures at which foodborne disease bacteria could grow. Roasts seldom cooled fast enough to comply with the requirement that foods cool from 60 to 7.2 °C (140 to 45 °F) in 4 h (9). They were usually within this range for several hours.

Rapid cooling is one of the most important critical control procedures for roast beef operations. Rapid cooling of large roasts, however, is difficult. Fortunately, the surfaces which contain most of the bacterial contaminants cool more rapidly than the interior portions, but even they do not cool rapidly when roasts are stored in pans containing other recently cooked or warmed roasts. One procedure that this study found to speed cooling was to wrap roasts and store them singly on shelves or in pans as opposed to putting them with other roasts in pans with lids. When practicable, roasts can be cut into (1/4 lb.) portions to reduce their bulk, wrapped in foil, and put into refrigerators (4). Where available, walk-in refrigerators rather than reach-in or cart (push-in) refrigerators should be used for cooling cooked roasts.

Reheating procedures were frequently such that foodborne pathogens would have survived if they had been present. With some of the hot-storage practices and the typical cooling practices, large numbers of foodborne disease bacteria, if the roasts were so contaminated, would develop on the roasts because of multiplication. Reheating, as cooling, is a critical point in the prevention of foodborne illness that might otherwise result from preparing and serving roast beef in foodservice operations. It is the last step in the operation that can also be a preventive measure.

Two approaches can be taken for reheating. One is for roasts that have been rolled, that have been boned and tied back together; it is also for roasts that have had thermopins or needles (for tenderizing) thrust into them, roasts that might have lymph nodes in the interior portions, and roasts that have been fabricated of chunk and ground meat. The second is for whole cuts of beef.
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<th>Product/cooling situation</th>
<th>Raw weight (pounds)</th>
<th>Probe position</th>
<th>Holding period (min)</th>
<th>Initial temperature (F)</th>
<th>Final temperature (F)</th>
<th>Minutes at 80-115°F</th>
<th>Minutes at 70-115°F</th>
<th>Minutes at 60-122°F</th>
<th>Minutes at 45-140°F</th>
<th>Potential for growth of foodborne disease bacteria</th>
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<td>+</td>
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<td>123</td>
<td>48</td>
<td>152</td>
<td>282</td>
<td>430</td>
<td>720</td>
<td>++</td>
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<td>260</td>
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<td>560</td>
<td>730</td>
<td>+++</td>
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<tr>
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<td>960</td>
<td>127</td>
<td>49</td>
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<td>175</td>
<td>295</td>
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<td>S</td>
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<td>139</td>
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<td>260</td>
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1 S = surface; SJ = just under surface; GC = geometric center; 3/4, 1 1/2 = distance in inches in roast; 2 = unknown weight; approximate weights sometimes given in parentheses. 4 = indicates expected multiplication of foodborne disease bacteria; + indicates expected multiplication reaching large numbers; ++ indicates expected multiplication reaching very large numbers (outbreak potential); +++ indicates no expected multiplication. 4 Roast fabricated from small chunks of beef and ground beef.
The surface of the roasts. Several surface-penetrating foodborne disease, even though the product will not be subjected to internal contamination, is to pasteurize to be overcooked.

Reheated roast beef, and dishes containing roast beef, are outbreaks because of reheating failures. So, these frequent ly identified as vehicles in foodborne disease recommend ations should be carefully followed to prevent temperature (such as 73.9°C served, and not in bulk as reheated roasts, a high assures rapid destruction of vegetative food borne disease bacteria must be used as an endpoint of reheating. Reheated roast beef, and dishes containing roast beef, are frequently identified as vehicles in foodborne disease outbreaks because of reheating failures. So, these recommendations should be carefully followed to prevent foodborne disease, even though the product will sometimes be considered from a merchandising standpoint to be overcooked.

The second approach, for whole cuts of beef that are subjected to internal contamination, is to follow recommendations of Bryan and Kilpatrick (4). These include cutting the roast into portions, wrapping them in foil, and reheating the portions until they reach a temperature of at least 71.1°C (160°F). Roasts can also be cut up and made into stews, beef stroganoff, and similar dishes, or ground for chili or tacos. During cutting or grinding, however, the meat can be subjected to further contamination. So, it is essential that these meat dishes be reheated to internal temperatures of at least 73.9°C (165°F). Because such dishes are sometimes in small portions or immediately served, and not in bulk as reheated roasts, a high temperature (such as 73.9°C; 165°F or higher) that assures rapid destruction of vegetative foodborne disease bacteria must be used as an endpoint of reheating. Reheated roast beef, and dishes containing roast beef, are frequently identified as vehicles in foodborne disease outbreaks because of reheating failures. So, these recommendations should be carefully followed to prevent foodborne disease, even though the product will sometimes be considered from a merchandising standpoint to be overcooked.

The second approach, for whole cuts of beef that are not subjected to internal contamination, is to pasteurize the surface of the roasts. Several surface-penetrating procedures have been found effective. They are — (a) immerse whole roasts in boiling stock or reheat roasts in stock that reaches boiling during heating (Table 7; Fig. 15); (b) heat foil-wrapped whole roasts or slices of roasted meat for sufficient time (usually an hour or longer) in ovens with temperatures of at least 325°F (Table 7; Fig. 12); (c) heat whole roasts or sliced, individual portions of meat in boiling-water-heated steam tables that have sliding covers over the insert pan and perforated bottom pans for sufficient time (usually an hour or longer; Table 8; Fig. 13); (d) immerse slices of roast in hot (92.8 to 100°C; 199 to 212°F) juss (Table 8; Fig. 17); (e) heat slices of beef in enclosed steam-injection steamers (Table 8; Fig. 16). Any of these procedures, properly carried out, can effectively pasteurize surfaces of roasts. They will not pasteurize the deep internal portions of the roast and frequently will not even warm such portions. Thus, the reheated meat can still be offered as medium rare or as cold slices.

Figure 18 illustrates the preparation of roast beef that was involved in an outbreak of C. perfringens gastroenteritis. It emphasizes points of contamination, opportunities for survival during cooking and reheating, and the potential for growth during cold storage, reheating, and hot storage. Similar situations are repeated with too frequent regularity.

Because of the frequency with which roast beef is implicated as a vehicle in foodborne disease outbreaks, it is essential that the foodservice industry take appropriate action to safely prepare and store roast beef. Unfortunately, cooked meat is considered by many to be safe and subsequent handling is no cause for concern. Managers of establishments that serve roast beef must be informed (in training courses, during inspections and consultations, or in foodservice journals, or via other media) of the hazards associated with improper roast beef handling, hot storage, cooling, and reheating practices and how to carry out these procedures safely. Health department personnel will have to provide appropriate consultation, training, and surveillance. Only after these procedures are instituted as routine practice in roast beef operations will this product cease to be a vehicle of foodborne illness.

ACKNOWLEDGMENTS

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REFERENCES

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<th>Minutes at 150-164.9°F</th>
<th>Minutes at and above 165°F</th>
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<th>Minutes at 60-105°F</th>
<th>Minutes at 106-122°F</th>
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TABLE 8. Time-temperature exposure of surface and internal portions of cooked beef roasts during reheating.

Note: a. Potential for survival of foodborne bacteria is based on the time-temperature exposure of the food and the type of bacteria present.
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<th>Barbecued Meat in Steamer</th>
<th>Unwrapped Roast in Oven</th>
<th>Foil-Wrapped Roast in Oven</th>
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1. S = surface; Sb = surface bottom; Sbd = surface bottom in depression; Sft = surface top on fat; Ss = surface side; Sms = surface top under muscle sheath; Ssp 1/2 = surface partially in meat; Snb = surface next to bone; Ssf = surface top after fat removed; Sc = surface side in crevice caused by gristle; Sj = just under surface; GC = geometric center; 1/8, 3/4, 1/2 inches into meat; U = unrecorded weights.
2. + indicates expected survival; - indicates expected destruction of large numbers of vegetative foodborne disease bacteria.
3. Data omitted whenever fractions of minutes were involved.
4. ++ indicates expected multiplication of foodborne disease bacteria reaching large numbers; - indicates no expected multiplication.
Figure 15. Temperatures of a 12.25-lb. roast while being reheated in jus.

Figure 16. Temperature of the approximate geometric center of a 2-oz. packet of sliced, cooked, barbecued beef while being heated in a small, hand-operated steamer.


Figure 17. Temperatures of surface and internal locations of a 7.5-oz. slice (9/16 to 1 1/4 inches thick) of prime-rib of beef while being dipped into hot jus.

Figure 18. Possible time-temperature relationships during preparation of roast beef that was the vehicle in a *Clostridium perfringens* gastroenteritis outbreak.


Fate of Spoilage Microorganisms in Frozen and Chilled Concentrated Tomato Juices

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Division of Food Technology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50-200, Israel

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ABSTRACT

Survival of four potential spoilage organisms was studied in tomato concentrates (22°, 28° and 34° Brix) stored at +7, 0 and -18 C for 4 months. In concentrates stored at -18 C, viable counts of Lactobacillus plantarum remained practically stable whereas those of Leuconostoc mesenteroides declined rapidly during the storage period, its death rate being reduced as the juice concentration was increased. Viable counts of Candida krusei and even more so of Torulopsis holmii, decreased progressively during frozen storage. In concentrates held at 0 C, viable counts of the four test organisms decreased during storage, regardless of the juice concentration, but at +7 C, for three out of the four test organisms, the ability to spoil the concentrates was dependent on the juice concentration.

Spoilage of tomato canned products has been investigated generally in connection with spore-forming bacteria (9, 10, 11). However, spoilage of these products by lactic acid bacteria and yeasts is not rare in the industry and results, in most instances, from recontamination of the processed cans during the cooling and post-cooling stages.

At present there is a relatively small amount of published data regarding the behavior of non-spore-forming bacteria and yeasts in tomato concentrates. In spite of the increasing prevalence of aseptic techniques in bulk filling, storage and transportation of "commercially-sterile" concentrated tomato juices, these products - in many processing plants all over the world - are still being filled into barrels and stored either refrigerated or frozen until they are canned for marketing. Such products may become contaminated and spoil when improper handled before canning.

These studies were made to determine the changes in counts of potential spoilage lactic acid bacteria and yeasts in tomato concentrated juices during a 4-month period, as affected by the juice concentration and the temperature of storage.

MATERIALS AND METHODS

Organisms and culturing conditions

Two lactic acid bacteria and two yeasts, isolated in our laboratory from spoiled tomato concentrates, were selected for use in this study: Lactobacillus plantarum TL-6, Leuconostoc mesenteroides TL-15, Torulopsis holmii TY-11 and Candida krusei TY-13. Bacterial inocula were prepared from 18-h-old cultures in APB broth at 30 C (4); yeast inocula were prepared from cells grown on potato dextrose agar (PDA, Difco) slants, incubated for 7 days at 30 C (5).

Tomato preparations

A 36° Brix commercially-sterile tomato concentrate was mixed with inocula prepared in amounts of sterile distilled water calculated to yield 34°, 28° and 22° Brix juices. Non-inoculated juices were also prepared. These mixtures, prepared in amounts of 250-350 g in sterile polyethylene bags, were homogenized for 2 min, after partial evacuation, with a Stomacher 400 homogenizer (A. J. Seward Co., Ltd., London). The percentages (w/w) of the 36° Brix concentrate used in the preparation of the 34°, 28° and 22° Brix juices were 94.5, 78.0 and 61.0%, respectively.

Seventeen-ml sterile polystyrene pots with polyethylene snap-on lids were filled aseptically with approximately 20 g of inoculated or non-inoculated juices and immediately stored at +7, 0 or -18 C. Less than 1/4 h were required to equilibrate the temperature of the juices (measured with thermocouples at the center of the container) with those of the storage cabinets.

Microbial counts

Individual containers were taken, after selected storage periods, and analyzed for both microbial counts and odor changes. The contents of each container were mixed thoroughly with a sterile plastic paddle and a sample of 3-4 g was transferred to a sterile 20-ml beaker, weighed accurately, and then diluted with two additional parts (by weight) of distilled water. Appropriate decimal dilutions of this mixture were prepared with 0.3 M sucrose, 1.0-ml samples plated on CA agar and the plates incubated at 30 C for 2-7 days. Log counts were calculated per g of concentrate, but no correction was made for the fact that with the more viscous juices the samples plated were actually smaller than the 1.0 ml taken in the pipette. Each container, once analyzed, was discarded.

The CA medium used throughout this study for enumeration of both lactic acid bacteria and yeasts consisted of the following (per liter of medium): Bacto-tryptone, 10 g; Bacto-yeast extract, 5 g; D-glucose, 10 g; sucrose, 10 g; NaH2PO4•2 H2O, 4 g; citric acid, H2O, 2 g; tri-potassium citrate, H2O, 10 g; MgSO4•7 H2O, 0.6 g; MnCl2•4H2O, 0.1 g; agar, 17 g; final pH, ca. 5.5. Whenever one of the test organisms was not recovered from a stored sample with the CA medium, that sample was further analyzed after it had been incubated for up to 3 weeks at 30 C. In such instances, analyses were done also using PDA for yeasts and APT agar (6) for the lactic acid bacteria.

pH and a° determinations

The pH was measured with a Radiometer PHM26 pH meter (Radiometer A/S, Copenhagen, Denmark). Equilibrium relative
humidity measurements of tomato concentrates were made at 20 C with a Hygro sensor element (no. 4-4837; Hygrodynamics, Inc., Silver Spring, Md.) mounted in lids of 8-oz. jars. The hygrometer sensor was attached to a Hygrometer indicator (model no. 15-3030 E; Hygrodynamics, Inc.) and an equilibrium time of not less than 24 h was allowed before measurements were made from 90-ml portions of the sample tested. Sensor calibrations were done against a saturated KNO₃ solution.

Soluble solids
A Bausch and Lomb refractometer was used to determine the soluble solids. Results are expressed as degrees Brix at 20 C.

Titratable acidity (TA)
Ten g of concentrate samples was mixed with 90 ml of distilled water and the resulting mixture was titrated with 0.1 N NaOH to pH 8.1. The results are reported as milliequivalent acid per kg concentrate.

Sensory evaluations
Immediately after sampling for microbiological analysis, the juices were tempered to 20 C and then checked for odor by a panel of three judges. Uninoculated samples stored at -18 C were used for comparison. Scores from 0 (no off-odor) to 4 (strong off-odor) were used in scoring the samples; samples with total scores of 8 or more points were considered spoiled.

RESULTS
In preliminary tests 250-g portions of a 36° Brix concentrate were “stomached” with 15 ml of a suspension of either L. plantarum or C. krusei in distilled water. After 2-min of “stomaching” aerobic plate counts, made on samples taken from five different places in the Stomacher bag contents (two replicates), gave the following average values ± standard deviation (log CFU/g): 6.60 ± 0.046 for L. plantarum and 5.26 ± 0.033 for C. krusei.

The tomato concentrates used in this study had the following characteristics: 34° Brix - 0.95 aW, 4.10 pH and 365 meq acid/kg TA; 28° Brix - 0.96 aW, 4.10 pH and 300 meq acid/kg TA; 22° Brix - 0.97 aW, 4.12 pH and 235 meq acid/kg TA.

Survival curves for L. plantarum and L. mesenteroides in the tomato concentrates stored at -18 C are shown in Fig. 1. Little or no change in viable counts of L. plantarum was observed during the first 90 days of storage, regardless of the juice concentration. Between 90 and 120 days L. plantarum counts declined to an extent of up to ca. 1.5 log cycles. A steady decline in the numbers of colony-forming units (CFU) of L. mesenteroides with storage time at -18 C was observed. During the first 10-day period the numbers of viable L. mesenteroides decreased by 0.3, 1.5 and 2.4 logs; between 10 and 30 days a further decrease in the numbers of CFU by 1.5, 2.1 and 4.7 logs occurred for 34°, 28° and 22° Brix concentrates, respectively. After 30 days of storage in 22° Brix concentrate and 60 days in 28° or 34° Brix concentrate, L. mesenteroides was not recovered if the stored samples were analyzed directly. However, this organism was recovered if the samples were incubated for 1-3 weeks at 30 C before analysis. L. mesenteroides was not recovered from 22° Brix samples stored for 60 days or from 28° or 34° Brix samples stored for 90 days even after pre-incubation.

The number of viable yeasts declined progressively with time in concentrates stored at -18 C (Fig. 2). During 120 days of storage, viable counts of C. krusei decreased by 1.3 to 2.0 logs, whereas counts of T. holmii declined by 4.7 and 3.5 logs within 80 days in 34° and 22° Brix concentrates, respectively. At the end of the 120-day storage period, T. holmii could not be recovered from any of the concentrates stored at -18 C.

In concentrates stored at 0 C, little change in counts of L. plantarum, L. mesenteroides, and C. krusei was observed within the first 20-day storage period, all logs of CFU being at least 92% of those measured at zero time (Fig. 3 and 4). A marked decline in counts of these three organisms occurred between 20 and 120 days of storage. On the other hand, counts of T. holmii decreased during the first 20-day period by 2.6 to 3.2 logs, continued to decline, and at 40 days in 22° and 28° Brix concentrates and 80 days in 34° Brix concentrate, this organism could not be recovered any more. Spoilage off-odors were not detected in any of the samples stored at -18° or 0 C.
Growth and survival curves for the lactic acid bacteria and yeasts in tomato concentrates stored at +7 °C are illustrated in Fig. 5 and 6. An increase in counts of *L. plantarum* occurred in 22° and 28° but not in 34° Brix concentrate, whereas an increase in counts of *L. mesenteroides* occurred only in the 22° Brix concentrate.

Viable counts of *C. krusei* increased with time in the three juice concentrations tested. In 28° and 22° Brix concentrates after 10 days of storage the number of CFU of *C. krusei* increased by 1.1 and 1.3 logs, whereas in 34° Brix concentrate a 1.3 log increase occurred only after 30 days of storage.

On the other hand, viable counts of *T. holmii* declined during storage of the concentrates at +7 °C; within the first 30-day period they decreased by 0.8 to 3.4 logs. Although increase in count was not detected in the 22° Brix concentrate, it had spoilage off-odors at the tenth day of storage. In samples inoculated with *L. plantarum*, spoilage off-odors were apparent after 8 days in 22° Brix and 15 days in 28° Brix concentrates. In samples of 22° Brix inoculated with *L. mesenteroides*, spoilage was detected after 20 days of storage. In samples inoculated with *C. krusei*, spoilage off-odors were detected after 10 days of storage in 22° and 28° Brix and after 40 days in the 34° Brix concentrate.

**DISCUSSION**

Concentrated tomato juices (22°, 28° and 34° Brix) were artificially inoculated with *L. plantarum*, *L. mesenteroides*, *T. holmii* and *C. krusei*. The Stomacher homogenization technique used for these preparations had the following advantages: satisfactorily uniform inoculation of the thick tomato concentrates was obtained, and excessive aeration of the juice was avoided.
Frozen storage

Survival curves obtained in tomato frozen concentrates for L. plantarum were significantly different from those of L. mesenteroides.

Counts of L. plantarum remained practically stable throughout the 120 days of storage of the concentrates at -18°C, but some reduction in counts was observed during the last 30-day period. Counts of L. mesenteroides, on the other hand, declined progressively with time, and during the first 30-day period had an average death rate of 0.4 to 1.4 log CFU/g per week. After 30 days in 22°C Brix and 60 days in 28°C and 34°C Brix concentrates, the L. mesenteroides population consisted mostly of dead cells; however, part of the population apparently consisted of sublethally-injured cells, as they could be recovered after incubation of the frozen-thawed samples at 30°C. L. mesenteroides could not be recovered after 60 days of frozen storage in 22°C Brix and 90 days in 28°C and 34°C Brix concentrates. It is demonstrated that the death rate of L. mesenteroides in frozen storage is lowered upon the increase of the juice concentration. In commercial varieties of tomatoes, the free sugars in the pulp of the red fruit are almost entirely glucose and fructose, while sucrose rarely exceeds 0.1% of the fresh weight (2). The increase in glucose and sucrose in the juice, resulting from concentration, could explain an increase in the protective effect against frozen storage injury to microorganisms (7).

The survival of lactic acid bacteria in frozen and chilled acid food products has been widely investigated in connection with orange concentrates (1, 8). The 0.95 aw found for 34°C Brix tomato concentrate approaches the aw value of 30°C Brix orange concentrate (5). Murdock and DuBois (8) showed that in a 42°C Brix orange concentrate (pH 3.75), viable counts of Lactobacillus and Leuconostoc decreased progressively at -17.8°C, with less than a one-log reduction within 60 days of storage.

Yeasts are considered to be very resistant to low temperatures and able to survive frozen storage; however, they may be injured when frozen in a acid medium (6). The number of viable yeast cells declined progressively with time in the frozen tomato concentrates, C. krusei being, however, much more resistant to freezing injury than T. holmii.

Chilled concentrates

Viable counts of the four test organisms decreased during storage of the concentrates at 0°C. Particularly sensitive at this temperature was T. holmii; viable counts of this yeast decreased during the first 30 days at an average rate of 0.7 to 0.8 log CFU per week.

As opposed to the results observed at 0°C, the four test organisms were able to grow and cause spoilage in some of the concentrates stored at +7°C. Growth at +7°C of the two strains of lactic acid bacteria studied was found to depend upon the degree of juice concentration: counts of L. plantarum increased during storage in 22°C and 28°C Brix concentrates, whereas those of L. mesenteroides increased only in the 22°C Brix concentrate. C. krusei grew in the concentrates tested, regardless of the juice concentration. On the other hand, T. holmii was unable to grow in 28°C and 34°C Brix concentrates; in the 22°C Brix concentrates spoilage occurred after 10 days of storage, although no growth of this organism was observed.

Berry et al. (1) reported that strains of Lactobacillus sp. and Leuconostoc sp. did not grow in 20°C (pH 3.90) or 30°C Brix (pH 3.80) orange concentrates stored at 4.4°C. At 10°C, growth of these organisms was observed in 20°C Brix orange concentrates, but their counts remained practically unchanged during 14 days of storage in 30°C Brix concentrates.

From the data reported herein, it appears that rapid chilling followed by refrigerated storage at ca. 0°C can, as efficiently as frozen storage, prevent microbiological spoilage of tomato concentrates for at least 4 months.

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Quality Aspects of Commercial Frozen Minced Fish Blocks

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ABSTRACT

Imported frozen minced Alaska pollock blocks were inferior in flavor and texture when compared to minced blocks prepared from certain species of North Atlantic fish. Organoleptic scores were strongly influenced by the cooking method, that is, baked versus fried. Quality attributes assessed by objective tests (thiobarbituric acid, trimethylamine, dimethylamine, aerobic plate count) generally did not show strong correlation with sensory scores.

Practically all frozen minced fish blocks used by commercial fish processors in this country are imported. There is very little, if any, production within the continental United States (21). By the time these blocks are converted into consumer products such as breaded sticks and portions, a great deal of the frozen shelf life of the blocks may have been expended as a result of the total elapsed time in storage contributed by the primary manufacturer before releasing for sale, transit time during shipment, and by the secondary processor before conversion into finished products. Several researchers have shown that minced fish has a shorter frozen storage life than intact fillets (4, 19). This is primarily due to the disintegration of the cellular integrity which enhances denaturation of proteins and loss of texture. In addition, development of oxidative rancidity is intensified, thus creating a problem with certain species of fatty fish. Protein denaturation not only affects the textural quality of breaded sticks or portions made from the affected blocks, but also causes a loss in functional property (binding) which is an important characteristic for preparing heat-gelled products (26). Although these storage changes in frozen minced fish can be suppressed considerably at low, subfreezing temperature (3, 18, 26), in commercial practice it is currently not feasible to maintain the low holding temperature usually required [≤-20 F (-29 C)].

During the early 1970s, some seafood processing companies suffered financial losses through marketing frozen minced seafood products which rapidly deteriorated in quality in retail distribution channels with subsequent consumer rejection. Use of marginal quality blocks (Alaska pollock) may also have contributed to this situation in some instances (23).

Dyer (17) proposed that standards of quality for minced fish blocks be directed to inform the secondary processor of the inherent stability of the particular frozen mince so that suitable finished products with a reasonable shelf life could be manufactured.

In a previous paper, results of a survey of the microbiological quality of commercial frozen, minced fish blocks were reported (20). This communication reports the quality of those blocks as measured by organoleptic and chemical tests.

MATERIALS AND METHODS

A total of 208 frozen minced fish blocks weighing either 16.5 or 18.5 lb. (7.5 or 8.4 kg) were analyzed in this survey. The source of these blocks and the species of fish involved were described in a previous publication (20).

Flavor and texture evaluations were conducted with a 12-member, experienced panel using a 9-point scale with descriptive terms ranging from "excellent" to "inedible" and in which a score of 5 represents a judgement of "borderline." USDC inspectors occasionally participated on the panel. Samples were prepared for tasting by wrapping small portions (2.5 × 2.5 × 5 cm) of the frozen block in foil and baking in an oven at 350 F (176.7 C) for 20 min. Some of the samples were also tasted as precooked breaded sticks after heating in an oven from the frozen state.

Trimethylamine (TMA) and dimethylamine (DMA) were estimated by the method of Castell et al. (12). This particular procedure was selected because of the convenience in simultaneously assaying both amines; however, it is acknowledged that the dithiocarbamate method for measuring DMA is probably more accurate. Results were expressed as mg of TMA or DMA nitrogen/100 g of flesh.

The method of Yu and Sinnhuber (25, 30) for determining thiobarbituric acid (TBA) number was modified by adding EDTA and propyl gallate during extraction (8).

Each block was sampled in duplicate while frozen using an electric drill with a 1-½-inch (32 mm) high-speed bit. Analyses made on both samples were averaged, and that figure was reported as the value for the block.

The procedure for aerobic plate count (APC) was described in a previous paper (20).

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Linear regression analyses were done on the data using a Wang Series Advance Programming Calculator.

RESULTS AND DISCUSSION

The percent frequencies of flavor and texture scores of the various minced blocks prepared by baking are in Tables 1 and 2. In accordance with the organoleptic scoring system employed, a numerical rating of just less than 5 would be indicative of unacceptability. Thus, from the data it can be estimated that 92% of the Alaska pollock, 29% of the cod, 33% of the cod frame, 21% of the pollock (N. Atlantic), and none of the haddock blocks were unacceptable because of undesirable flavor; whereas, 100%, 40%, 21%, 19% and 44% of the same respective species were unacceptable because of poor texture. Textural degradation was manifested principally as toughness or rubberiness, whereas flavor change was usually characterized as soapy, bitter or fishy.

TABLE 1. Percent frequency of flavor scores for various frozen minced fish blocks.

<table>
<thead>
<tr>
<th>Flavor score</th>
<th>Alaska pollock</th>
<th>Cod</th>
<th>Cod frame</th>
<th>Pollock</th>
<th>Haddock</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0-1.9</td>
<td>1.5</td>
<td>5.4</td>
<td>3.1</td>
<td>24.5</td>
<td>43.7</td>
</tr>
<tr>
<td>2.0-2.9</td>
<td>7.7</td>
<td>43.1</td>
<td>7.7</td>
<td>31.1</td>
<td>22.2</td>
</tr>
<tr>
<td>3.0-3.9</td>
<td>24.3</td>
<td>37.1</td>
<td>33.3</td>
<td>69.2</td>
<td>55.6</td>
</tr>
<tr>
<td>4.0-4.9</td>
<td>20.8</td>
<td>20.8</td>
<td>19.2</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>5.0-5.9</td>
<td>66.7</td>
<td>66.7</td>
<td>66.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0-6.9</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0-7.9</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No. blocks 65 70 24 26 9

TABLE 2. Percent frequency of texture scores for various frozen minced fish blocks.

<table>
<thead>
<tr>
<th>Texture score</th>
<th>Alaska pollock</th>
<th>Cod</th>
<th>Cod frame</th>
<th>Pollock</th>
<th>Haddock</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0-1.9</td>
<td>46.1</td>
<td>43.1</td>
<td>37.1</td>
<td>20.4</td>
<td>22.2</td>
</tr>
<tr>
<td>2.0-2.9</td>
<td>24.3</td>
<td>24.3</td>
<td>33.3</td>
<td>69.2</td>
<td>55.6</td>
</tr>
<tr>
<td>3.0-3.9</td>
<td>37.1</td>
<td>20.8</td>
<td>69.2</td>
<td>55.6</td>
<td></td>
</tr>
<tr>
<td>4.0-4.9</td>
<td>20.8</td>
<td>19.2</td>
<td>22.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0-5.9</td>
<td>66.7</td>
<td>66.7</td>
<td>66.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0-6.9</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0-7.9</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The rationale for tasting the samples baked was that this is the culinary method employed by the U. S. Department of Commerce inspectors in grading fish blocks; also, it had been demonstrated that this particular cooking method allowed for better discrimination among samples of frozen fish which had undergone storage deterioration (16). However, it was realized that frying usually masks or subdues off-flavors (7); since the consumer would ultimately taste the finished product made from these blocks as a fried batter-breaded item, a number of the blocks, just for comparison, were also tasted in this manner in addition to the baked form. Linear regression analysis done on the data plotted as baked flavor score (Y) as a function of fried flavor score (X) yielded a regression line of: \( Y = 1.04 X - 1.23 \), with a correlation coefficient of 0.87. Similarly, the relationship for baked texture score as a function of fried texture score was: \( Y = 0.94 X - 0.49 \), with a correlation coefficient of 0.89. In both instances the average fried flavor or texture score was about one scale unit higher than the corresponding baked score. The average flavor and texture scores and standard deviations (o) are compared in Table 3 for the various minced blocks tasted as baked and fried fish. The higher rating of fried samples is readily apparent. From a consumer product standpoint, a greater percentage of blocks would have been considered acceptable after frying than was estimated from baked scores. To exemplify this point, whereas 40%, 21% and 44% of the cod, pollock and haddock blocks were judged unacceptable in the baked form due to either poor flavor or texture, 8% of the cod blocks, but none of the pollock or haddock blocks, were regarded as unacceptable when tasted as fried (breaded) products. This discrepancy in quality grading due to the method of culinary preparation is a controversial issue with some seafood processors.

In addition to assessing the quality of frozen minced fish by sensory methods, another objective of this study was to determine whether or not this parameter could be measured by certain chemical indices of spoilage. The average TBA number, TMA and DMA contents and standard deviations for the various minced blocks are in Table 4. The TMA value has been employed as a chemical index of the quality of the fish before freezing (14). In the present investigation, cod frame mince had the highest average level of TMA. This is not surprising in view of the additional handling that is involved with frames, and that unwashed mince from frames usually contains a large amount of blood pigments which reduce the naturally occurring precursor, TMA oxide, to TMA (9). Mince from pollock, a species with much dark muscle, was next highest in average TMA content.

TABLE 3. Comparison of baked and fried average flavor and texture scores of various minced fish blocks.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. blocks</th>
<th>Baked</th>
<th>Avg. flavor score</th>
<th>Fried</th>
<th>Avg. texture score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Avg. flavor score</td>
<td></td>
<td>Avg. texture score</td>
</tr>
<tr>
<td>Cod</td>
<td>45</td>
<td>5.7</td>
<td>0.9</td>
<td>6.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Pollock</td>
<td>26</td>
<td>5.4</td>
<td>0.5</td>
<td>6.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Haddock</td>
<td>9</td>
<td>5.6</td>
<td>0.6</td>
<td>6.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Cod frame</td>
<td>6</td>
<td>5.9</td>
<td>0.8</td>
<td>6.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Alaska pollock</td>
<td>5</td>
<td>2.8</td>
<td>0.6</td>
<td>3.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Hake</td>
<td>4</td>
<td>2.8</td>
<td>0.6</td>
<td>7.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Ocean catfish</td>
<td>3</td>
<td>6.3</td>
<td>0.6</td>
<td>7.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Ling cod</td>
<td>3</td>
<td>4.9</td>
<td>0.6</td>
<td>5.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

1 Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.
Based on organoleptic evaluation, Dyer and Dyer (15) found a TMA value above 15 to be indicative of spoiled or spoiling fish. Twenty-five percent of the pollock or cod frame blocks exceeded this value. However, it cannot be stated unequivocally that these blocks were produced from poor raw material since it was previously indicated that heme pigments in pollock or cod frame mince catalyze formation of TMA; also, it has been demonstrated that the process of mincing caused an immediate increase in TMA value (5). Results of chemical tests for quality should be interpreted cautiously with minced fish because if the mince has been washed, there can be a drastic reduction in TMA content (13) and in volatile acids and volatile bases (27).

It is believed by some researchers that the "fishy" odor of stale or spoiling fish is due to the presence of TMA. In this study, there was some correlation between flavor score and TMA content for cod frame blocks (r = 0.68) and pollock blocks (r = 0.63), but not much (< 0.5) for cod or Alaska pollock blocks. Thus, it is not considered that the TMA test would be reliable indicator of the quality of frozen minced fish blocks. No significant correlation was found between flavor score and APC at 21°C for blocks from any of the different species.

Sinnhuber and Yu (25) found poor quality frozen fish to have TBA numbers of 4 or greater. Since none of the blocks examined in this study exceeded a TBA number of 3.5, it is concluded that oxidative rancidity as measured by this parameter was not a major source of the off-flavors in those blocks that were rated low. Miyauchi et al. (22) reported minced Alaska pollock to have relatively good stability against development of rancid flavors during frozen storage; yet, in their storage studies with this species, flavor deterioration still occurred.

It has been suggested that the concentration of DMA in certain species of frozen fish could be used as a measure of frozen storage deterioration since it was found to correlate with the development of toughening (10, 29). This compound has been detected in frozen Alaska pollock (28) and in other gadoid species (11) with the order of increasing activity being haddock, cod, pollock, cusk and hake. Dimethylamine and formaldehyde are both formed in equimolar amounts from the enzymatic degradation of TMA-oxide, but it is actually the formaldehyde that induces textural toughening through denaturation of myofibrillar proteins (I). In this survey the Alaska pollock blocks were relatively high in average DMA content; this seems to reflect the low texture scores afforded these particular blocks. Arithmetic means for DMA content of the North Atlantic species were low with the exception of hake which ranged in content from 23 to 36 mg/100 g for four blocks. To the authors' knowledge, there are no published data relating concentration of DMA with textural quality of frozen fish. Linear regression analysis was done on the data (baked texture scores as a function of DMA content) to ascertain the degree of correlation and the value of DMA corresponding to borderline texture. Although some correlation was found (r ranged from -0.41 to -0.58), it was not considered sufficiently significant to validate the application of this test for grading texture of frozen minced blocks of unknown history. Sikorski et al. (24) have reviewed the mechanisms of protein denaturation in fish. Although formation of formaldehyde (and DMA) may be of some significance in the textural degradation of frozen gadoid fish, other reactions may also occur and contribute to the overall toughening process during frozen storage.

In general, this limited survey indicated imported frozen commercial minced Alaska pollock blocks to be of inferior quality compared to minced blocks made from certain species of North Atlantic fish with the possible exception of hake. The principal cause of quality failure was textural change which was described as rubbery, spongy, or fibrous. Although some blocks received low flavor scores this defect could be obviated to some extent through addition of flavoring or seasoning agents in the finished product as occurred when the mince was tasted as fried breaded fish sticks. A defect in texture would preclude use of the afflicted blocks for production of breaded sticks or portions; however these blocks could still be used to produce fish cakes or other "hash" type products which contain other texture modifying ingredients.

The minced Alaska pollock blocks examined in this survey are called "surimi" in Japan, and this product is tailored specifically as a frozen raw material for kamaboko (22). A desirable characteristic of kamaboko is a chewy or resilient texture (2) which might be undesirable in a fish stick or portion to an American consumer. Bond (6) does not recommend frozen surimi as a raw material for breaded sticks or portions. However, this product was the only Alaska pollock mince that was available during the survey period to U.S. seafood processors who were not fully cognizant of its storage characteristics and who were principally attracted to it because of its low cost and abundance.

It was surprising to find a rather low level of DMA in cod frame mince since this material may contain blood
and kidney tissue which are known to accelerate DMA formation. Perhaps, the known susceptibility of this product to storage deterioration promotes expeditious usage so that the blocks we examined were relatively young. Nevertheless, since frozen minced fish is more prone to quality loss than intact fillets, it would behoove the fish processor to consider the age of the block and also the fish species in his decision on the final end product to be manufactured, as suggested by Dyer (17), and also to aim for a rapid turnover of the product in retail trade.

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The authors acknowledge the technical assistance of M. E. Haskins and W. S. Hill.

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Effect of Inorganic Nutrients on Production of Steroid Glycoalkaloids by Phytophthora infestans Race 1.2.4 In Vitro

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ABSTRACT

The influence of sodium salts of the macronutrients nitrate, phosphate, sulfate, and chloride; and the micronutrients iron, calcium, copper, cobalt, nickel, and manganese on growth of Phytophthora infestans and synthesis of glycoalkaloids by the fungus was investigated. Maximum growth levels were demonstrated when 0.04% phosphate, 0.04% chloride, or 2-5 mg of iron/liter were employed in the culture medium. Results indicate that upon substitution of the individual sodium salts of the macronutrients for the potassium and magnesium salts or addition of sodium chloride to the basal medium, the concentration of glycoalkaloids synthesized by the fungus decreased significantly. Regression analysis showed that the concentration of phosphate in the medium had the most influence on the amount of glycoalkaloids produced by P. infestans. Increasing the phosphate concentration in the medium resulted in increasing amounts of glycoalkaloids being produced by the fungus. As the mass of mycelium increased in media containing different amounts of phosphate, the quantity of glycoalkaloids synthesized by the fungus decreased.

The basic nutritional requirements of phytopathological fungi include carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur as structural components and enzyme constituents. Of the metallic elements, plant pathogens need relatively large amounts of potassium and magnesium with trace amounts of iron, zinc, copper, manganese and molybdenum (9). Due to trace quantities of microelements necessary for fungal growth it is difficult to determine the requirements of these nutrients. Most investigators who culture P. infestans add no micronutrients to their media (3,5,12). Cuppett and Lilly (2) reported that addition of 1.0 mg of ferric iron/liter with 200 mg of ascorbic acid/liter to a medium containing glucose and asparagine yielded excellent growth of P. infestans.

Steroid glycoalkaloids are bitter tasting, toxic substances that occur in normal Irish potatoes in trace amounts. The toxicity of these compounds has been demonstrated in animals and fungi. Considerable literature is available that cites cases of potato poisoning in animals with gastrointestional and neurological disturbances. The glycoalkaloid level in potatoes has been shown to increase substantially upon infection with P. infestans (6,7). Accumulation of alkaloids in blighted potatoes has been attributed to an increase of physiological stress in the potato due to infection with P. infestans.

Since steroid glycoalkaloids were found to be synthesized by P. infestans in an earlier study by Maas et al. (8) this study was undertaken to determine the response of P. infestans to a spectrum of mineral nutrients and the effect of these chemicals on synthesis of glycoalkaloids by the fungus.

MATERIALS AND METHODS

The organism used in this study was P. infestans race 1.2.4 (8). The control medium was the enriched basal medium developed by Maas et al. (8) which consisted of: 90.0 g of maltose, 20.0 g of L-proline, 20.0 g of DL-alanine, 1.0 g of KNO3, 0.5 g of KH2PO4, 0.25 g of MgSO4 ·7H2O, and 1.0 mg of thiamin per liter of distilled water. Sodium salts of nitrate, phosphate and sulfate were individually substituted for the potassium and magnesium salts in the control medium. Also sodium chloride and sodium acetate were individually added to the basal medium. Table 1 shows the nutrients and respective concentration ranges used in this study. Trace elements were tested individually in the concentration range of 0.0001 - 5.0 mg/l and included calcium, cobalt, copper, iron, manganese and nickel. Double distilled water and analytical grade chemicals were used in the portion of the investigation dealing with micronutrients.

All media (50-ml volumes in 250-ml Erlenmeyer flasks) were inoculated with 0.5 ml of a standardized mycelium suspension. The mycelium was harvested and filtered through preweighed Whatman #2 filters. The filters and the mycelium were air-dried to a constant weight and mycelium dry weight/50 ml of medium was calculated. Glycoalka-

| TABLE I. Concentration range of the sodium salts utilized in the enriched basal medium. |
|-----------------|-----------------|-----------------|
| Macronutrient   | Concentration range (%) |
| NaNO3          | 0.025 - 0.5      |
| NaH2PO4        | 0.001 - 0.4      |
| Na2SO4         | 0.001 - 0.2      |
| NaCl           | 0.001 - 0.4      |
| Na(C2H3O2)     | 0.001 - 0.4      |

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oids were extracted, separated by ammonia precipitation which was followed by color development of the steroid with sulfuric acid and formaldehyde and quantitation spectrophotometrically using the method described by Gull and Isenberg (4) with modifications by Wu and Salunkhe (18). Details of the above methods have been described previously (8).

RESULTS

In the medium containing sodium salts of the macronutrients none of the mycelium yields was greater than the yield in the control (basal medium) (Fig. 1). Mycelium production in the medium containing sodium salts was lower than mycelium yield in the basal medium; however, the greatest amounts of growth detected in media including sodium salts were not significantly lower at the 0.05% statistical level than the amount detected in the control medium. The greatest amounts of growth in the media containing sodium salts were observed when the following were used: 0.025-0.10% NaNO3; 0.02-0.04% NaH2PO4; 0.02-0.05% Na, 0.2% NaCl; and 0.01-0.04% Na2SO4. Sodium acetate appeared to be inhibitory to P. infestans since all mycelium yields were significantly lower than the amount of mycelium in the basal medium.

**Figure 1.** Growth of Phytophthora infestans race 1.2.4 after 28 days at 21°C in enriched basal medium containing individually substituted sodium salts of the macronutrients in the concentration range of 0.001 - 0.5% (w/v) with 95% confidence limits.

Addition of micronutrients to the basal medium gave varied results (Fig. 2). The amount of mycelium detected in the control medium in the micronutrient study (Fig 2) was smaller than the mycelium yield of the control medium in the macronutrient study (Fig. 1). This may be attributed to a decrease in the amount of contaminating microelements due to use of glass double-distilled water and analytical grade chemicals in the control medium for the micronutrient study. Growth of the fungus was stimulated by addition of iron in the ferrous state with significantly higher levels of P. infestans obtained when iron was added to the medium in the concentration range of 1.0-5.0 mg/l. P. infestans race 1.2.4 growth was significantly inhibited by all concentrations of copper, cobalt, nickel, manganese, and by concentrations of calcium that exceeded 0.001 mg/l. All results are reported as an average of three replicates with each replicate including 10 culture flasks and statistical analysis was done at the 95% level.

**Figure 2.** Growth of Phytophthora infestans race 1.2.4 after 28 days at 21°C in enriched basal medium containing nitrate salts of micronutrients in the concentration range of 0.0001 - 5.0 mg/l with 95% confidence limits.

Synthesis of glycoalkaloids by P. infestans race 1.2.4 in the media containing sodium salts is illustrated in Fig. 3. The average level of glycoalkaloids detected in the control medium was 0.85 mg of total glycoalkaloids/25 ml of medium. Upon substitution of sodium salts for the individual potassium and magnesium salts of the macronutrients or addition of sodium chloride, the average concentration of glycoalkaloids decreased significantly. Although variations in the quantity of glycoalkaloids produced in the media containing sodium salts were observed, these differences were not significant at the 95% statistical level. To determine the effect of each sodium salt on synthesis of glycoalkaloids by P. infestans, simple regression analysis was done on (a) the effect of nutrient concentration and (b) the effect of

**Figure 3.** Quantity of glycoalkaloids synthesized by Phytophthora infestans race 1.2.4 after 28 days at 21°C in enriched basal medium containing sodium salts of the macronutrients individually substituted for the potassium and magnesium salts with 95% confidence limits.
growth level of *P. infestans* on the quantity of glycoalkaloids synthesized by the fungus. Results of the analysis indicate that the concentration of NaNO₃ or NaCl in the media had little effect on the amount of glycoalkaloids produced by the fungus. In media containing NaH₂PO₄ or Na₂SO₄, as their concentrations were increased the level of glycoalkaloids synthesized by *P. infestans* increased in accordance with the equation $y = 0.1616 + 0.0396X$ and $y = 0.73 + 0.0362X$, respectively, where $y = \text{mg of total glycoalkaloids per 25 ml of medium and } X = \text{concentration of macronutrient in percent}$. Analysis of results relating the amount of glycoalkaloids produced by the fungus to the mycelium yield in media containing various concentrations of sodium salts of the macronutrients showed that in various concentrations of NaCl the yield of mycelium had little effect on the quantity of glycoalkaloids synthesized by the fungus. But when a range of concentrations of NaH₂PO₄ or Na₂SO₄ were used in the media, the quantity of glycoalkaloids produced by the fungus decreased as the amount of growth increased in relation to the linear equations, $y = 0.1278 - 0.224X$ and $y = 0.1919 - 0.3224X$, where $y = \text{mg of total glycoalkaloids per 25 ml and } X = g \text{ of dry weight mycelium per 50 ml}$. When *P. infestans* was grown in media containing different concentrations of NaNO₃, the glycoalkaloid concentration increased as the amount of growth of *P. infestans* race 1.2.4 increased according to the equation $y = 0.1582 + 0.0602X$ where $y = \text{mg of total glycoalkaloids per 25 ml and } X = g \text{ of dry weight of mycelium per 50 ml of medium}$. Analysis of all positive samples from the control medium and the other test media containing sodium salts of the macronutrients was done by thin layer chromatography. Results of this analysis show that the basal medium contained both solanidine, present in all samples, and solanine, present in 20% of the samples. In media containing sodium salts only solanidine was present.

No glycoalkaloids were detected in the test media containing copper, cobalt, calcium, nickel or manganese. Upon addition of iron to the basal medium, glycoalkaloids were found to be produced by *P. infestans* at all amounts of iron except 5.0 mg/1. Figure 4 illustrates the concentration of glycoalkaloids synthesized by the fungus in media containing various concentrations of iron. The highest concentration of glycoalkaloids was obtained when 0.001 mg of iron/liter was added to the medium. Statistical analysis of these data indicates that the amount of glycoalkaloids produced in the basal medium was significantly greater than the amounts detected in the media containing iron, although in media containing 0.001 or 0.01 mg of iron/liter the amount of glycoalkaloids synthesized by *P. infestans* was significantly greater than the quantity of alkaloids produced in media containing greater amounts of iron. Regression analysis illustrates that as the concentration of iron increased the level of glycoalkaloids synthesized by the fungus declined in accordance with the linear equation $y = 3.341 - 7.819X$ where $y = \text{mg of total glycoalkaloids per 25 ml of medium and } X = \text{concentration of iron in mg/1}$. As various concentrations of iron were added to the medium, the amount of glycoalkaloids produced by the fungus decreased as the mycelium yield increased. This relationship of these parameters is shown by the simple regression equation $y = 0.4462 - 0.428X$ where $y = \text{mg of total glycoalkaloids per 25 ml of medium and } X = g \text{ of dry weight of mycelium per 50 ml of medium}$.

**DISCUSSION**

Since *P. infestans* has been a parasite of the Irish potato for a long time, one would expect its nutritional requirements to be related to the chemical composition of the potato. The raw Irish potato contains per 100 g of wet weight: 17.6 g of carbohydrate, 2.1 g of protein, 0.1 g of fat, 407 mg of potassium, 53 mg of phosphate, 7 mg of calcium, 3 mg of sodium, 0.6 mg of iron, and a total ash content of 900 mg (13). The results indicate that *P. infestans* responded well to phosphate and chloride at the 0.04% level with a decrease in mycelium yield observed when these nutrients were increased to the 0.05% level. This suggests that the 0.05% phosphate level used in the basal medium may be somewhat inhibitory to the fungus. The maximum amount of mycelium obtained when Na₂SO₄ was used in the medium is considerably lower than the maxima obtained with other macronutrients. This may be due to the lack of magnesium in the medium rather than an influence of sulfate since magnesium is important in cell metabolism as a cofactor for many enzymes and in ribosomal function.

Stimulation of growth of *P. infestans* by the greater amounts of iron tested in this study (2-5 mg/1) may be attributable to enhancement of activity of heme proteins and other oxidative enzymes which use iron as a cofactor. Apparently the requirements of *P. infestans* for calcium, copper, cobalt, nickel, and manganese are lower than 0.0001 mg/1 since at this amount and above some degree of inhibition of *P. infestans* was observed. These data...
indicate that an enriched medium for culturing *P. infestans* should include: 1.0 g of KN0₃, 0.4 g of KH₂PO₄, 0.25 g of MgSO₄·7H₂O and 2-5 mg of ferrous iron per liter of distilled water.

Glycoalkaloids, members of a group of compounds known as secondary chemicals that occur in plants and microorganisms, are not known to serve any direct physiological function (I). Most of these chemicals are not waste products, but rather are secondary substances produced by active synthesis. These compounds often contain nitrogen or other elements which are available to organisms in limited supply and it requires energy to synthesize secondary chemicals. Synthesis of glycoalkaloids by the potato is induced when the plant has been subjected to physiological stress, such as infection by *P. infestans*.

Our results indicate that synthesis of glycoalkaloids by *P. infestans* race 1.2.4 is dependent upon the chemical composition of the medium, as illustrated by the significant decrease in the quantity of glycoalkaloids synthesized by the fungus when sodium salts were used in the medium. The enzyme systems involved in the synthesis of alkaloids are probably adapted to the chemical composition of the potato which contains a potassium to sodium ratio of approximately 400:1. In microorganisms, potassium has been shown to function in the cell as a compatible solute for enzyme stability (I); therefore disruption of the potassium: sodium ratio of the cell may result in decreased activity of one or more of the enzymes involved in synthesis of steriod glycoalkaloids. Of the macronutrients tested, it appears that the concentration of nitrate or chloride has little effect on the enzymes involved in synthesis of steriod glycoalkaloids. With increasing concentrations of phosphate or sulfate in the medium, the quantity of glycoalkaloids produced by the fungus increased; this could be attributable to a higher degree of stress applied to the fungus by greater concentrations of these nutrients. The fungus may respond to a more stressful environment in the same manner as the potato, i.e. by production of more stress metabolites, such as steriod glycoalkaloids. Phosphate appears to have the most influence on the relationship between the amount of mycelium produced and the amount of glycoalkaloids synthesized by the fungus. With the spectrum of phosphate concentrations used in this study, as the amount of mycelium increased the quantity of glycoalkaloids synthesized by the fungus declined. This resultant relationship may be due to an adjustment in metabolism of the fungus from synthesis of glycoalkaloids to synthesis of factors which relate directly to growth. This shift in metabolic pathways may be due to the easing of stressful conditions when larger masses of mycelium are present.

The results show that the greatest glycoalkaloid production in media containing iron occurred with a low level of iron (0.001 mg/1) and was followed by a decline in the quantity of glycoalkaloids synthesized by *P. infestans* as the concentration of iron in the medium increased. This phenomenon has been demonstrated in the synthesis of other toxic substances by microorganisms. Pappenheimer et al. (II) found that when the iron concentration reached 100 μg/1 synthesis of toxin by *Corynebacterium diptheriae* reached its maximum and decreased as the amount of iron was increased to 500 μg/1, where it became negligible.

**REFERENCES**

Organochlorine Insecticides and Industrial Pollutants in the Milk Supply of Southern Ontario, Canada — 1977

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ABSTRACT

During the summer of 1977 milk samples were collected from 308 bulk transporters picking up milk in the southern region of Ontario. These milk samples were subjected to a comprehensive analysis for organochlorine insecticides and selected halogenated industrial pollutants. Ninety-five percent of samples contained residues of p,p'-DDE, dieldrin, heptachlor epoxide and PCB, 68% contained residues of HCB, and 16% contained residues of p,p'-DDE and p,p'-TDE; chlordane and endosulfan were detected in only a few samples. Residues of aldrin, endrin, lindane, methoxychlor, mirex, PBB and chlorinated phenols were not detected. Mean residues of insecticides in milk fat were 0.015 ppm; dieldrin, 0.011 ppm; heptachlor epoxide, 0.002 ppm; endosulfan, 0.001 ppm; and chlordane, <0.001 ppm. Mean residues of industrial pollutants in milk fat were 0.03 ppm PCB and 0.02 ppm HCB. Compared with a similar survey conducted in 1973 for an approximately identical geographical area, levels of p,p'-DDT, PCB, and HCB showed a significant decrease, dieldrin showed only a slight decrease and heptachlor epoxide and chlordane increased. Reports are presented on individual case histories involving the misapplication of endrin, lindane, and fenthion to dairy cattle. The rate of elimination of these compounds from milk is presented.

Residues in bovine milk are a convenient indicator for reflecting the pattern and volumes of use of organochlorine compounds in the agricultural industry and milk quality is frequently used as a basis for legislative decisions concerning changes in the permitted uses of pesticides. Most of the agricultural and industrial activity in Ontario is concentrated in the southern region and previous milk surveys for this region have been conducted (4,5). Both the provincial and federal governments have been involved in restricting and removing registered uses of persistent organochlorine compounds for agricultural and non-agricultural purposes (Table 1). Beginning in 1969 with the removal of all agricultural uses of aldrin and dieldrin, and continuing to 1978 with removal of chlordane, the organochlorine insecticides have gradually been replaced by less-persistent alternative compounds. While aldrin and dieldrin were used primarily in agriculture, chlordane and DDT have found wide use in rural, urban, and recreational areas. Compounds such as HCB and PCB have no significant agricultural uses but are widely used in a variety of industrial applications. Changes in uses of pesticides are governed by the Pesticide Act of Ontario and by the Pest Control Products Act of Canada. Industrial compounds such as polychlorinated biphenyls are now being controlled under the Environmental Contaminants Act of Canada and the Environmental Protection Act of Ontario. The volumes of past agricultural uses of pesticides in southern Ontario have been reported by Frank et al. (3) and uses of PCB are documented in a joint report by Environment Canada and Health and Welfare Canada (2).

The present survey was undertaken to establish current levels of persistent organochlorines in the milk supply from the major production area in Ontario, to observe the effects of the restrictions and removals placed on various insecticide uses during the 1967-1977 period, and to check for the presence of other halogenated compounds not sought in previous surveys but which are known to be present in the Great Lakes basin.

MATERIALS AND METHODS

Sampling procedure

Milk samples collected in the survey were obtained from 308 bulk transporters hauling milk from 11 southern Ontario counties (Table 2, Fig. 1). The milk in each transporter represented the accumulation of 2-day milking from 5 to 20 farms or 7,000 to 18,000 kg of fluid milk. Collections were made by field personnel of the Milk Industry Branch, Ontario Ministry of Agriculture and Food, and delivered promptly to the Provincial Pesticide Residue Testing Laboratory for analysis. Sample size approximated 500 ml and glass jars were used for transport.

Samples were forwarded to the Central Milk Testing Laboratory, Ontario Ministry of Agriculture and Food for determination of milkfat content. Fat determinations were made according to official turbidimetric procedures (7).

All 308 samples were analyzed for residues of diphenylethanes, cyclodienes, lindane, mirex, and polychlorinated biphenyls (PCB). All samples from Brant and Wentworth counties plus every 10th sample from the remainder of the region were analyzed for hexachlorobenzene
TABLE 1. Chronological restrictions and removals of organochlorine compounds from uses in Ontario under the Ontario Pesticide Act, the Pest Control Products Act and the Environmental Contaminants Act, 1969-1978.

<table>
<thead>
<tr>
<th>Year</th>
<th>Compound</th>
<th>Agriculture &amp; Forestry</th>
<th>Domestic</th>
<th>Industrial &amp; structural</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969</td>
<td>Aldrin</td>
<td>all uses halted by midsummer</td>
<td>all uses halted by end of year</td>
<td>restricted to termite control</td>
</tr>
<tr>
<td></td>
<td>Dieldrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heptachlor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>DDT</td>
<td>general uses halted: licensed use on tobacco, vegetables, and apples</td>
<td>all uses halted</td>
<td>restricted to bat and mouse control</td>
</tr>
<tr>
<td></td>
<td>TDE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1971</td>
<td>PCB</td>
<td>licensed use on vegetables halted</td>
<td></td>
<td>voluntary removal of open system uses</td>
</tr>
<tr>
<td>1971</td>
<td>DDT</td>
<td>licensed use on tobacco halted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td>DDT</td>
<td>label uses on tobacco halted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td>Endrin</td>
<td>licensed use on apples and potatoes halted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1973</td>
<td>DDT</td>
<td>licensed use on apples halted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td>HCB</td>
<td>registered use not renewed recommendations for use on tobacco removed</td>
<td>no domestic uses</td>
<td>legislative controls on use restrictions to use in electrical transformers and capacitors</td>
</tr>
<tr>
<td>1975</td>
<td>Endosulfan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1976</td>
<td>PCB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1977</td>
<td>Chlor dane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1977</td>
<td>Endosulfan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1978</td>
<td>Chlor dane</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Incidence of organochlorines in milk.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. All 308 samples analysed for:</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>97.1</td>
</tr>
<tr>
<td>p-p'-TDE</td>
<td>16.0</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>16.6</td>
</tr>
<tr>
<td>o,p'-DDT</td>
<td>0.3</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>0.0</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.0</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.0</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>99.3</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.0</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>99.3</td>
</tr>
<tr>
<td>cis &amp; trans Chlordane</td>
<td>4.2</td>
</tr>
<tr>
<td>Oxychlorane</td>
<td>0.0</td>
</tr>
<tr>
<td>a &amp; b Endosulfan</td>
<td>1.0</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>7.5</td>
</tr>
<tr>
<td>PCB</td>
<td>94.5</td>
</tr>
<tr>
<td>Mirex</td>
<td>0.0</td>
</tr>
<tr>
<td>B. 81 samples analysed for:</td>
<td></td>
</tr>
<tr>
<td>HCB</td>
<td>67.9</td>
</tr>
<tr>
<td>C. 71 samples analysed for:</td>
<td></td>
</tr>
<tr>
<td>PBB</td>
<td>0.0</td>
</tr>
<tr>
<td>D. 45 samples analysed for:</td>
<td></td>
</tr>
<tr>
<td>Chlorinated phenols</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1 All samples from Brant and Wentworth counties plus every 10th sample from remainder of region.
2 All samples from Essex county plus every 10th sample from remainder of region.
3 Included all 308 samples.

Figure 1. Map of southern Ontario showing the eleven counties included in the milk survey.

until residue of contaminants dropped below permissible or detectable levels in the milk.

Analytical procedures - insecticides. PCB, PBB, HCB and mirex

Pesticides were extracted from whole milk (100 g) by blending at high speed for 5 min with 200 ml of acetonitrile. A 50-g sample was removed by filtering; this was transferred to a separatory funnel, and partitioned by shaking for 30 sec with 100 ml of hexane. The acetonitrile phase was diluted with 300 ml of 1% aqueous sodium chloride solution and the mixture was shaken again for 30 sec. After phase separation, the aqueous extract was discarded and the hexane partitionate was washed once by shaking for 15 sec with 100 ml of water. The washed hexane partitionate was then concentrated to 5-10 ml with rotary vacuum after filtration through anhydrous sodium sulfate.

Cleanup was effected on Florisil according to the procedure of Mills et al. (8). The concentrated hexane extract was quantitatively transferred to a 22-mm I.D. chromatography column containing 25 g of Florisil (60/100 mesh, activated commercially at 650°C and stored at 135°C) and eluted successively with 200 ml 1:4-dichloromethane:hexane: (Fraction A) and 200 ml 0.35:50:50 - acetonitrile:dichloromethane:hexane (Fraction B). Eluates were concentrated to dryness with rotary
Vacuum; fraction A was re-dissolved in ca. 5 ml of acetone in preparation for separation of PCB, PBB, and HCB from lindane, chlordane, DDT analogs, and mirex: fraction B was re-dissolved in a measured quantity of iso-octane and contained heptachlor epoxide, dieldrin, endrin, methoxychlor, and the endosulfan analogs.

Separation of PCB, PBB, and HCB from lindane, chlordane and the DDT analogs was done by the procedure described by Holdren et al. (7). Coconut charcoal (50/200 mesh, Fisher #5-690), previously washed with acetone, dried, and stored at 135 °C, was placed in a 10-mm i.d. column to a height of 7.5 cm and sandwiched between two 1-cm layers of sand. The column was then pre-washed with 1:3-acetone:diethyl ether (v/v). The acetone solution of fraction A was introduced to the charcoal column and insecticides and mirex were eluted with 180 ml of 1:3-acetone:diethyl ether, PCB and PBB were eluted off the column with 80 ml of benzene, and PCB and HCB was removed by elution with 100 ml of toluene. Eluate fractions were concentrated to dryness with rotary vacuum and re-dissolved in measured amounts of iso-octane.

The cleanup and fractionation procedures differed from the methods reported in the previous surveys by Frank et al. (4,5) so as to include the analysis of previously unreported compounds, to improve detection capabilities, and to facilitate identification by GLC.

**Analytical procedure - chlorinated phenols**

Chlorinated phenols were determined using a procedure developed in this laboratory by Sirons (2). Whole milk was percolated through a column of Amberlite XAD-2 resin, 20/50 mesh, to isolate the chlorinated phenols. Description of the phenols was accomplished by elution with a 1:4 mixture of 0.1 N NaOH (aq)-methanol. The volume of methanol was reduced by rotary evaporation and the chlorinated phenols were then partitioned into chloroform, evaporated to dryness and methylated with diazomethane followed by cleanup on activated Florisil.

**Gas-liquid chromatography**

All compounds, with the exception of chlorinated phenols, were determined by electron capture gas chromatography using a 24Ni source which was operated in the pulsed and linearized mode. Organochlorine insecticides, PCB, and PBB were chromatographed on a 1.8 m x 2 mm i.d. column packed with 1.5% OV-17/2.0% OV-210 on 100-120 mesh Gas Chrom Q with a nitrogen carrier flow of ca. 60 ml/min and isothermal column temperature of 190 °C. PCB determinations were made on a 1.8 m x 2 mm i.d. column packed with 3% OV-1 on 100/200 mesh Gas Chrom Q with isothermal operation at 240 °C. Sample injections were 5 μl, representing a sample equivalent of 50 mg on a whole milk basis.

Chlorinated phenols were determined by Coulson conductivity detection (halogen mode) using a 1.8 m x 4 mm i.d. glass column packed with 5% OV-17 on silanized Chromosorb W-AW, 80/100 mesh. Injection volumes represented a 50-ng sample equivalent.

**Recoveries and detection limits**

Recoveries were checked periodically by direct fortification of whole milk with a acetone solution of known concentration of halogenated hydrocarbons. These samples were stored under refrigerated conditions for 2-3 days with occasional shaking, and then analyzed according to the described procedures. Mean recovery values and approximate limits of detection are listed in Table 3. Limits of detection are expressed at ppb in whole milk since the method was based on whole milk analysis rather than the direct analysis of milkfat. Detection limits on a fat basis, therefore, are dependent on and will vary directly as the variation in milkfat content.

By convention, all residue results in this report are expressed as ppm in milkfat and are derived by arithmetical conversion from residues in whole milk. The data presented in this report are not corrected for recovery losses.

**Confirmatory techniques**

Confirmation of residue identity was done on random samples and was achieved primarily with the use of two-dimensional thin layer chromatography (TLC), whereby the appropriate areas, after development, were scraped off the TLC chromatogram, re-dissolved in a desorbing solvent, and then re-examined by GLC. Some degree of confirmation was achieved as the result of the semi-specific nature of the cleanup and fractionation techniques and, alternatively, by alternate column GLC.

A few samples of milk were found which contained relatively high residues of DDT and endosulfan. In these samples confirmation was achieved by alkaline treatment for conversion to the dehydrochlorinated analogs for DDT and by examination using the sulfur-specific FPD detector for endosulfan.

**RESULTS AND DISCUSSION**

Over 95% of milk samples analyzed contained measurable residues of p,p'-DDE, dieldrin, heptachlor epoxide and PCB, and approximately two-thirds of samples analyzed contained detectable levels of HCB; p,p'-DDT and p,p'-TDE were found in only about one-sixth of samples. Endosulfan and chlordane were detected only occasionally while residues of aldrin, lindane, methoxychlor, mirex, PBB, and chlorinated phenols were not found above their respective detection limits (Table 3).

**TABLE 3. Average recoveries and approximate detection limits.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Recovery</th>
<th>Detection limits (ppb in milk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p,p'-DDT</td>
<td>80</td>
<td>0.1</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>90</td>
<td>0.1</td>
</tr>
<tr>
<td>p,p'-TDE</td>
<td>90</td>
<td>0.1</td>
</tr>
<tr>
<td>o,p'-DDT</td>
<td>87</td>
<td>0.1</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>84</td>
<td>2.0</td>
</tr>
<tr>
<td>Lindane</td>
<td>83</td>
<td>0.1</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>91</td>
<td>0.1</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>83</td>
<td>0.2</td>
</tr>
<tr>
<td>Endrin</td>
<td>80</td>
<td>0.5</td>
</tr>
<tr>
<td>cis-Chlordane</td>
<td>85</td>
<td>0.1</td>
</tr>
<tr>
<td>trans-Chlordane</td>
<td>82</td>
<td>0.1</td>
</tr>
<tr>
<td>Oxychlordane</td>
<td>81</td>
<td>0.1</td>
</tr>
<tr>
<td>α-Endosulfan</td>
<td>88</td>
<td>0.2</td>
</tr>
<tr>
<td>β-Endosulfan</td>
<td>82</td>
<td>0.5</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>87</td>
<td>1.0</td>
</tr>
<tr>
<td>HCB</td>
<td>75</td>
<td>0.01</td>
</tr>
<tr>
<td>PCB</td>
<td>80</td>
<td>0.5</td>
</tr>
<tr>
<td>PBB</td>
<td>90</td>
<td>0.5</td>
</tr>
<tr>
<td>Mirex</td>
<td>75</td>
<td>0.1</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>85</td>
<td>0.1</td>
</tr>
<tr>
<td>2,3,4,6-Tetrachlorophenol</td>
<td>80</td>
<td>1.0</td>
</tr>
<tr>
<td>2,4,5-Trichlorophenol</td>
<td>80</td>
<td>1.0</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>80</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Agricultural chemicals**

**ΣDDT.** The incidence of detectable p,p'-DDT and p,p'-TDE in milk samples was 16.6% and 16.0%, respectively; however, residues in most were only slightly higher than the detection limit of 0.002 ppm in milkfat; o,p'-DDT was detected in only one milk sample which contained a ΣDDT level of 0.27 ppm (Table 2, 4, 5).

p,p'-DDE was present in 97% of samples at a mean residue of 0.012 ppm in milkfat. In 51% of the bulk transporters ΣDDT ranged from 0.001 to 0.010 ppm and a further 32% ranged from 0.011 to 0.020 ppm. The highest concentration of DDE and ΣDDT originated in milk from Norfolk, a county which supports a large hectarage of tobacco and where DDT was used in large quantities until 1971; these tobacco soils have been found to still contain substantial residues of DDT (6).

The ΣDDT residues in milkfat as revealed in this survey show a significant decline from those reported in previous surveys (Fig. 2). With a sampling base covering a similar geographical area, the mean ΣDDT residues...
were 0.19 ppm and 0.5 ppm in milk fat, respectively for 1967-69 and 1973, as compared to a mean of 0.015 ppm \( \Sigma \text{DDT} \) in the present survey. This general downward trend in \( \Sigma \text{DDT} \) since the legislative restrictions in 1970-71 suggests a half-life biological disappearance for DDT from milk of about 2 to 4 years.

Based on the total annual milk production in the region, a calculated amount of 0.3 kg \( \Sigma \text{DDT} \) was present in the 1977 supply as compared to 1.1 kg present in 1973 supply, i.e., a drop of 0.8 kg (Table 6).

**Dieldrin.** Dieldrin was present in 99.3% of milk samples in the 1977 survey. Fifty-nine percent contained between 0.001 to 0.010 ppm in the milkfat and a further 35% contained from 0.011 to 0.020 ppm (Table 2,4,5). The highest residues were found in milk derived from those countries associated with tobacco and vegetable production, i.e., Elgin, Essex, and Norfolk (Table 5).

Dieldrin residues in milk declined only slightly between 1973 and 1977. As reported by Frank et al (5), in 1973 dieldrin concentrations in milkfat averaged 0.015 ppm in this region as compared to a 1977 mean value of 0.011 ppm. From the time that aldrin was removed from use in 1969, dieldrin in milk has declined at a half-life of approximately 5-6 years (Figure 2). Based on a total annual milk production for the region, 0.22 kg of dieldrin was present in the milk supply, a decline of 0.11 kg since 1973 (Table 6).

**Chlordane and heptachlor epoxide.** Chlordane has been increasingly used to control rootworm in corn following removal of aldrin, dieldrin, and heptachlor from agricultural uses in 1969 (5). Chlordane was not detected during the 1973 milk survey but was found in 4.2% of samples in the 1977 survey (Table 2). Residues consisted of cis- and trans-chlordane in an approximate 1:1 ratio; oxychlordane was not detected.

Chlordane formulations consist of a complex mixture of compounds. Cis- and trans-chlordane isomers, chlordene, nonachlor, and heptachlor are the major components (9); the heptachlor component can be present at 10% of the formulation. In the 1973 survey only 3.5% of milk samples contained detectable levels of heptachlor epoxide while in the 1977 survey, heptachlor epoxide was found in 99.3% of samples (Table 2). In 1973 the mean residue in milk fat was well below the 0.001 ppm detection limit while in 1977 the mean value was 0.004 ppm (Table 5). This increased incidence and concentration was attributed to the aforementioned increased use of chlordane (and the heptachlor component) in corn. Of the samples in which heptachlor epoxide was identified, 98.4% contained milkfat residues between 0.001 and 0.010 ppm (Table 4). Residues by county were similar across the entire sampling region, correlating with the wide-spread production of corn in all counties of southern Ontario.

**Endosulfan.** Endosulfan is formulated as a mixture of the \( \alpha \)- and \( \beta \)- isomers in an approximate ratio of 2:1 (10) and is rapidly converted to the sulfate and/or the diol metabolites following ingestion. Endosulfan sulfate was detected in 7.5% of milk samples in 1977 with an overall mean concentration of 0.002 ppm in milkfat (Table 2); in 1973 no endosulfan was detected in the milk from the region. Use of endosulfan has increased markedly since removal of DDT, particularly for the control of foliar insects on tobacco and vegetables. In 1975 official recommendation for its use on tobacco was deleted because of high residues appearing in the cured tobacco leaf (11). In those milk samples which contained endosulfan sulfate, 78% contained between 0.001 and 0.010 ppm.

Only two of 23 samples in which endosulfan was detected contained the \( \alpha \) or \( \beta \) isomer in addition to the sulfate metabolite. In one instance residues consisted of 0.12 ppm cis-endosulfan, 0.063 ppm trans-endosulfan and 0.004 ppm endosulfan sulfate. According to a report by Braun and Lobb (2), endosulfan is rapidly converted to the sulfate following ingestion by cattle. A ratio of 1:2:225 of the \( \alpha: \beta \):sulfate was reported in the milk 1 day after in accidental ingestion of the endosulfan formulation (2). Presence of the \( \alpha \)- and \( \beta \)-isomers in this single milk sample in an approximate ratio of 2:1

---

**TABLE 4. Frequency of organochlorine concentrations in milk fat samples.**

<table>
<thead>
<tr>
<th>Organochlorine</th>
<th>1</th>
<th>1-10</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-100</th>
<th>101-200</th>
<th>201-300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticide (308 analyses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>9</td>
<td>18</td>
<td>99</td>
<td>26</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>p,p'-TDE</td>
<td>259</td>
<td>45</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>o,p' &amp; p,p'-DDT</td>
<td>257</td>
<td>46</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>( \Sigma \text{DDT} )</td>
<td>9</td>
<td>148</td>
<td>90</td>
<td>30</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Dieldrin</td>
<td>2</td>
<td>180</td>
<td>108</td>
<td>16</td>
<td>2</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Chlordane</td>
<td>295</td>
<td>8</td>
<td>4</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Heptachlor epoxide</td>
<td>2</td>
<td>303</td>
<td>3</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Endosulfan</td>
<td>305</td>
<td>1</td>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>2</td>
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<td>0</td>
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<td>Endosulfan sulfate</td>
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<td>21</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>Industrial chemicals</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCB (81 analyses)</td>
<td>26</td>
<td>53</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PBB (71 analyses)</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCB (308 analyses)</td>
<td>17</td>
<td>25</td>
<td>34</td>
<td>66</td>
<td>69</td>
<td>38</td>
<td>47</td>
<td>12</td>
<td>0</td>
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<tr>
<td>Chlorinated phenols (45 analyses)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1Includes 2,4,6- and 2,4,5-trichlorophenols, 2,3,4,5-tetrachlorophenol and pentachlorophenol.
<table>
<thead>
<tr>
<th>County</th>
<th>1977 Production × 10^3 kg</th>
<th>% Fat</th>
<th>p,p-DDE</th>
<th>p,p-TDE</th>
<th>p,p-DDT</th>
<th>DDT</th>
<th>Dieldrin</th>
<th>Chlordane</th>
<th>Heptachlor epoxide</th>
<th>Endosulfan</th>
<th>HCB</th>
<th>PCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brant</td>
<td>33,696</td>
<td>3.56</td>
<td>0.015</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.018</td>
<td>0.011</td>
<td>0.001</td>
<td>0.003</td>
<td>0.005^1</td>
<td>0.003</td>
<td>0.032</td>
</tr>
<tr>
<td>Elgin</td>
<td>41,558</td>
<td>3.77</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td>0.016</td>
<td>0.024^2</td>
<td>0.021</td>
<td>ND</td>
<td>0.007</td>
<td>0.002</td>
<td>0.009</td>
<td>0.034</td>
</tr>
<tr>
<td>Essex</td>
<td>18,172</td>
<td>3.83</td>
<td>0.006</td>
<td>ND</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.015</td>
<td>ND</td>
<td>0.005</td>
<td>ND</td>
<td>0.003</td>
<td>0.035</td>
</tr>
<tr>
<td>Haldimand</td>
<td>50,387</td>
<td>3.82</td>
<td>0.007</td>
<td>&lt;0.001</td>
<td>ND</td>
<td>0.009</td>
<td>0.010</td>
<td>ND</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>ND</td>
<td>0.028</td>
</tr>
<tr>
<td>Kent</td>
<td>5,258</td>
<td>3.62</td>
<td>0.004</td>
<td>0.001</td>
<td>ND</td>
<td>0.005</td>
<td>0.010</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.001</td>
<td>ND</td>
<td>0.030</td>
</tr>
<tr>
<td>Lambton</td>
<td>32,940</td>
<td>4.34</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>0.007</td>
<td>ND</td>
<td>0.003</td>
<td>ND</td>
<td>0.002</td>
<td>0.059</td>
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<tr>
<td>Middlesex</td>
<td>94,069</td>
<td>4.09</td>
<td>0.013</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.014</td>
<td>0.006</td>
<td>ND</td>
<td>0.005</td>
<td>ND</td>
<td>0.002</td>
<td>0.038</td>
</tr>
<tr>
<td>Niagara</td>
<td>53,023</td>
<td>3.79</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>0.009</td>
<td>ND</td>
<td>0.002</td>
<td>0.001</td>
<td>0.002</td>
<td>0.033</td>
</tr>
<tr>
<td>Norfolk</td>
<td>14,031</td>
<td>3.70</td>
<td>0.027</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.029</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.018</td>
</tr>
<tr>
<td>Oxford</td>
<td>168,552</td>
<td>3.64</td>
<td>0.014</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.015</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.031</td>
</tr>
<tr>
<td>Wentworth</td>
<td>32,560</td>
<td>4.11</td>
<td>0.011</td>
<td>0.001</td>
<td>0.001</td>
<td>0.013</td>
<td>0.009</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
<td>0.002</td>
<td>0.044</td>
</tr>
</tbody>
</table>

| Totals or Weighted Mean | 544,246 | 3.83 | 0.012 | <0.001 | 0.002 | 0.015 | 0.011 | <0.001 | 0.004 | 0.001 | 0.002 | 0.035 |
| S.D.                  | 0.84     | 0.011 | 0.003 | 0.015 | 0.020 | 0.005 | 0.002 | 0.002 | 0.008 | 0.003 | 0.030 |

^1 Production data of milk supplied by the Ontario Milk Marketing Board, Toronto, Ontario (private communications).
^2 Includes one sample containing 0.23 ppm p,p-DDT, 0.025 ppm o,p-DDT, 0.007 ppm p,p-DDE and 0.005 ppm p,p-TDE.
^3 Includes one sample containing 0.11 ppm α-endosulfan, 0.063 ppm β-endosulfan, and 0.004 ppm endosulfan sulfate.
ND = Not detected.
< 0.001 = Unconfirmed trace.
and the relatively small amount of endosulfan sulfate indicated that both milks had been contaminated directly and did not result from the consumption of contaminated feed. A follow-up investigation failed to reveal the source of contamination.

**Endrin, lindane, and methoxychlor.** In the 1977 survey, no milk samples were found to contain residues of endrin, lindane, or methoxychlor. These compounds were also absent in the 1973 survey.

**Industrial contaminants**

**Hexachlorobenzene (HCB).** Eighty-one milk samples were analyzed for the presence of HCB and the compound was found to be present in 67.9% of these samples at a mean concentration of 0.002 ppm in the milkfat (Table 2.5). The incidence was similar to that of 69% reported by Frank et al. (5) in the 1973 survey but residues in 1973 were considerably higher at a mean concentration of 0.007 ppm in 1977. Only 20% of the bulk transporters were checked for HCB as compared to 42% in 1973.

**PCB concentrations in individual samples ranged from non-detectable to a high of 0.170 ppm in milkfat. On the basis of a mean value of 0.033 ppm, a total of 0.68 kg of PCB was calculated as being present in the current milk supply as compared to 2.33 kg in 1973 (Table 6).**

**Polychlorinated biphenyls (PCB).** In the 1977 survey 94.5% of milk samples were found to contain detectable levels of PCB, i.e., greater than 0.005 ppm in milkfat. The overall mean residue was 0.033 ppm as compared to 0.11 ppm in the 1973 survey (5). Mean residues by county ranged from 0.018 ppm to 0.059 ppm with the highest levels being observed in Lambton and the lowest in Norfolk (Table 5). No geographic pattern could be derived from these residues with respect to the distribution of PCB; current information indicates that PCB in the rural area may originate from local electrical transformers, rainwater, or fallout (5).

PCB concentrations in individual samples ranged from non-detectable to a high of 0.170 ppm in milkfat. On the basis of a mean value of 0.033 ppm, a total of 0.68 kg of PCB was calculated as being present in the current milk supply as compared to 2.33 kg in 1973 (Table 6).

**Polybrominated biphenyls (PBB) and mirex.** Neither PBB nor mirex was detected in the milk from the region in 1977. All bulk transporters were sampled and analyzed for mirex while only 23% were sampled for PBB analyses. In addition to the survey, several dairies in Essex county were sampled specifically for PBB but no positive samples (>0.5 ppm in milkfat) were found.

**Chlorinated phenols.** A total of 45 samples were analyzed for chlorinated phenols. These samples represented composites of 7 to 8 individual bulk transporters. Analyses included pentachlorophenol, 2,3,4,6-tetrachlorophenol, plus 2,4,5- and 2,4,6-trichlorophenol; none of these phenols were detected in milk down to a detection limit of 0.1 ppb in whole milk for pentachlorophenol and 1 ppb in whole milk for the trichlorophenols (Table 3).

**Case histories**

Three individual case histories were investigated in which endrin, lindane, and fenthion were accidentally applied as topical treatments for insect control on dairy cattle. Residues appeared almost immediately in the milk and shipments were temporarily terminated while the milk was monitored to establish the point in time at which shipments could be resumed.

**Endrin.** In August, 1976 a dairy herd was accidentally treated with endrin for control of flies. Poisoning symptoms developed rapidly in 25 animals with the subsequent death of one cow and one calf. A composite milk sample collected one day after the treatment contained endrin at a level of 0.40 ppm in whole milk (8.7 ppm in milkfat). Within 34 days this level had declined to 0.0026 ppm in whole milk (0.056 ppm in milkfat) to give a biological disappearance with a half-life of 4 to 5 days (Fig. 3). Milk from individual cows was analyzed on day

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**TABLE 6. Change in actual amounts of organochlorine in milk supply from southern region of Ontario.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Volume of Milk ( \times 10^3 ) kg</th>
<th>DDT</th>
<th>Dieldrin</th>
<th>HE &amp; Chlorodane</th>
<th>PCB</th>
<th>HCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973</td>
<td>660,559</td>
<td>1.1</td>
<td>0.33</td>
<td>Nil</td>
<td>2.33</td>
<td>0.152</td>
</tr>
<tr>
<td>1977</td>
<td>554,246</td>
<td>0.3</td>
<td>0.22</td>
<td>0.09</td>
<td>0.68</td>
<td>0.045</td>
</tr>
</tbody>
</table>
13 following the exposure and endrin levels ranged from 0.031 to 0.16 ppm in whole milk.

**Lindane.** Thirteen cows in a herd of 50 were accidentally treated with lindane in November, 1977. Attempts were made to remove the chemical as quickly as possibly by thoroughly washing the cows. Analysis of a composite milk sample from the 13 cows on the following day revealed lindane at 0.17 ppm in the whole milk. After 21 days this level dropped to 0.005 ppm. Biological disappearance with a half-life of 5 to 6 days was calculated (Fig. 3). Individual cows showed variations ranging from 2 to 6 days for the lindane to drop by 50%.

**Fenthion.** A dairy herd treated accidentally with fenthion produced milk with a residue of 0.042 ppm in a composite sample collected 1 day after the treatment. Eight days later the fenthion residue had dropped to 0.0046 ppm indicating a half-life disappearance period of 2 to 3 days.

**ACKNOWLEDGMENTS**

The authors acknowledge the efficient milk collection system provided by field staff of the Milk Industry Branch, Ontario Ministry of Agriculture and Food. The co-operation of the Central Milk Testing Laboratory for determination of milkfat in all milk samples is also hereby gratefully acknowledged. Thanks go to the technical staff of the Pesticide Laboratory for sample extraction.

**REFERENCES**

Evaluation of Sauerkraut-Like Products From Direct-Acidification of Cabbage

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ABSTRACT

Direct acidification of cabbage using acetic, citric, glucono-delta-lactone, or lactic acid was evaluated as a means of producing sauerkraut-like products. Direct acidification with citric acid gave the most acceptable product for incorporation into sauerkraut-containing foods, but none of the acids gave freshly-prepared products with sauerkraut-like flavor and odor. Sensory evaluations with laboratory panels showed that citric acid-acidified cabbage and naturally fermented sauerkraut were comparable when cooked with frankfurters or incorporated into a three-bean salad. Crisp texture and absence of strong odors were attributes of directly acidified cabbage that would be desirable for some applications of these products.

Development and acceptance of sauerkraut-like products from cabbage by direct acidification could lead to an expanded use of cabbage in contemporary foods, especially if unique properties can be identified. Other advantages of direct acidification over natural fermentation could include shorter production times, elimination or reduction of waste brine, and better control over process variables. Natural lactic acid fermentation of cabbage takes 3 to 4 weeks or longer, and involves a complex microbial succession (8, 9, 13). Although infrequently encountered in modern processing, pink, rancid, soft, or dark krauts occur from defective fermentations, and greatly reduce acceptability (6, 7).

A precedent for production of preserved vegetable products by direct acidification exists for processed dill pickles, sour pickles, or sweet pickles using salt stock (2, 7), and for fresh-pack dill pickles using cucumbers (3). Additional measures may be incorporated to extend shelf-life, and these include blanching, pasteurization, addition of preservation, and refrigeration. Short-term home preservation of cabbage by some ethnic groups is achieved by acidification along with refrigeration, and some limited commercial-scale production of acidified, shredded cabbage is currently practiced (5). The latter practice involves repacking in fresh brine at the time of distribution, and addition of either sorbic acid, sodium benzoate, or sulfur dioxide to retard spoilage and to retain a raw appearance in the finished product.

The purpose of this investigation was to prepare directly acidified cabbage products, and to evaluate their characteristics as foods.

MATERIALS AND METHODS

Product formulations

Initial formulations used in directly acidifying cabbage were based on the composition data (Table 1) of Pederson et al. (9) for sauerkraut. Five acidification systems were prepared and evaluated. One lot was prepared with a mixture containing lactic acid (85%) and acetic acid (glacial) which was added to shredded cabbage to result in a final calculated concentrations of 1.45% (w/w) lactic acid and 0.45% (w/w) acetic acid. A second lot was prepared with citric acid (crystalline monohydrate) which was added at a rate to give 1.5% (w/w) citric acid in the finished product. A similar lot was prepared except that lemon juice (Realemon®) was used to provide 1.5% of citric acid. Glucono-delta-lactone, a slow-release acid, was added to another lot to result in a final concentrations of 1.5% (w/w) acid as citric acid. Finally, a lot was prepared with a mixture of acetic, citric and lactic acids which were added at a level so that a final concentration of 0.75% (w/w) lactic acid, 0.25% (w/w) acetic acid and 0.50% (w/w) citric acid was achieved. Ethanol (95%) was included in all formulations to result in a 0.5% (w/w) concentration in the finished products. Sodium chloride was also added to all samples to give a final concentration of 2.0% (w/w).

Cabbage obtained locally was prepared by removing the outer leaves of each head, and then each was washed and cored. Cabbage was shredded by slicing at approximately ¼ inch intervals with an electric slicer, and this was followed by chopping resulting leaf strips into:

Additional measures may be incorporated to extend shelf-life, and these include blanching, pasteurization, addition of preservation, and refrigeration. Short-term home preservation of cabbage by some ethnic groups is achieved by acidification along with refrigeration, and some limited commercial-scale production of acidified, shredded cabbage is currently practiced (5). The latter practice involves repacking in fresh brine at the time of

<table>
<thead>
<tr>
<th>Component</th>
<th>Sauerkraut (%)</th>
<th>Sauerkraut juice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>0.78</td>
<td>0.69</td>
</tr>
<tr>
<td>Salt</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Total acidity</td>
<td>1.48</td>
<td>1.67</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.40</td>
<td>0.45</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.37</td>
<td>1.45</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 Adapted from Pederson et al. (9).
2 From Pederson (6).
approximately 1/4 inch lengths with a knife. After shredding, appropriate ingredients were added, and the mixtures were packed in closed polyethylene containers, and stored at 4 °C for at least 1 week before evaluations to allow product equilibration.

The effect of blanching upon the texture (12) of citric acid-acidified cabbage was investigated using a steam tunnel with the belt speed varied to give the desired blanch time. Blanching was done after the cabbage was shredded and acidified, but before equilibration in refrigerated storage. Product temperatures immediately after blanching were determined with a mercury thermometer. Freezing was also investigated as a method of altering the texture of the product. For this aspect citric acid-acidified cabbage samples were frozen at either -40 °C or -18 °C for 12 h, and this was followed by storage at 4 °C.

### Determination of sensory properties

Sensory evaluations of the products prepared in the initial phase were carried out by five experienced food professionals as informal, descriptive evaluations. The more detailed evaluations of the citric acid-acidified product were conducted under controlled laboratory conditions, and employed 24-29 panelists experienced in sensory evaluations of foods. Panelists were seated in individual booths in the laboratory, which was equipped with indoor fluorescent lighting (78 footcandles). Water was available for the judges to use at their own discretion.

Sauerkraut and frankfurter items were prepared with both naturally fermented commercial sauerkraut and citric acid-acidified cabbage. One kg of sauerkraut was combined with seven commercially prepared all meat frankfurters that were cut into 1-inch pieces. Samples were warmed to an internal temperature of 79 °C with a steam table before evaluations.

Three-bean salads were prepared with both citric acid-acidified cabbage and naturally-fermented sauerkraut. Three cups of sauerkraut were combined with ¾ cup of fine cut green peppers, one cup of fine cut fresh celery stalks, ¼ cup fine cut white onion, one can (16 oz) of red kidney beans, ¼ cup granulated white sugar and ½ tsp each of salt and pepper. Samples were equilibrated at 5 °C for 24 h.

Citric acid-acidified cabbage containing naturally produced sauerkraut juice was prepared by combining three cups of citric acid kraut and 1-1/8 cups natural kraut juice, and allowing equilibration at 5 °C for 24 h before evaluation. Both three-bean salad and plain sauerkraut items were removed from refrigeration (ca 5 °C) and served to panelists as chilled items. Although not identified to panelists, a naturally-fermented, canned, commercial sample of sauerkraut was included in sensory evaluation sessions for direct comparison of attributes.

For serving, 2 oz of each sample were portioned into sampling cups coded with three digit random numbers. Five-ounce clear plastic sampling cups were used for the warmed, sauerkraut-hot dog samples, while 4-oz. polyethylene-coated paper cups were used for the three-bean salad and the unheated samples.

The samples were evaluated with a quantitative descriptive analysis (11) ballot which included scales for intensity of acid flavor, intensity of sweetness, type of flavor, overall texture and overall preference. For statistical analysis of sensory data, individual descriptive scales on the ballots from each panel evaluation session were coded on a seven-point basis. A value of 1.0 was assigned to the extreme left end of each line, and a value of 7.0 was assigned to the extreme right end of each corresponding line. Each panelist’s marked judgments were then coded with the appropriate intermediate numerical values (30 mm per whole number with 3-mm increments).

Coded values from the ballots were punched into computer cards, and data were analyzed by the University of Wisconsin 1110 computer for analysis of variance appropriate for a randomized complete block design (10). Individual statistical analyses were done for each quality attribute of the samples evaluated in a panel session. Mean scores for each sample attribute, F-values for the whole comparison of sample attributes, and least significant differences (LSD) for attributes of each pair of samples were obtained (10) when a significant F-value was found for the whole comparison.

### Chemical analyses

Titratable acidity was determined by AOAC methods (1), and pH measurements were made with a Corning Products Model 12 Research pH Meter.

### RESULTS AND DISCUSSION

The titratable acidity and pH data for the initially prepared acidified products are in Table 2 along with those of the comparative naturally-fermented sauerkraut sample. From these data it is apparent that not all the added glucono-delta-lactone dissociated into gluconic acid. This acidulant gives a slow release of acid, and in the shredded cabbage system two weeks at 4 °C allowed only partial dissociation of glucono-delta-lactone. The data for the other samples shows that the pH and titratable acidity of the products approximated those of the naturally-fermented sauerkraut.

Samples acidified with acetic and lactic acid exhibited a tart taste which was followed by a slightly sweet aftertaste. This sweet aftertaste could have been due to the presence of sugars which normally would have been removed by metabolism of the fermenting microorganisms in the natural production of sauerkraut. The odor of this experimental sample was not pronounced or objectionable, but did not resemble that of sauerkraut. It was characterized as resembling that of acetic acid, e.g., slightly vinegar-like. A mixture of acetic, lactic, and citric acid resulted in a product with mildly acid taste, a noticeable cabbage-like flavor, and a sweet aftertaste. The odor was similar to that of the samples acidified individually with acetic and lactic acids. Glucono-delta-lactone yielded the least acceptable product of those prepared. The taste was only slightly tart, and a sweet aftertaste was noted along with a hint of putrefaction which could have been caused by the slow release of acid.

Acidification of cabbage with citric acid resulted in the most tart flavor of the acidulants used, and was even

### TABLE 2. Chemical characteristics of initial experimental products.

<table>
<thead>
<tr>
<th>Sample description</th>
<th>pH</th>
<th>meq/100 ml</th>
<th>Percent acid</th>
<th>Calculated acid addition (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Sauerkraut</td>
<td>3.50</td>
<td>15.0</td>
<td>1.35 (as lactic)</td>
<td></td>
</tr>
<tr>
<td>1.45% lactic acid &amp; 0.45% acetic acid</td>
<td>3.03</td>
<td>25.0</td>
<td>2.24 (as lactic)</td>
<td>2.18 (as lactic)</td>
</tr>
<tr>
<td>0.75% lactic, 0.25% acetic &amp; 0.5% citric</td>
<td>3.15</td>
<td>21.5</td>
<td>1.94 (as lactic)</td>
<td>1.88 (as lactic)</td>
</tr>
<tr>
<td>1.50% citric acid</td>
<td>2.95</td>
<td>23.5</td>
<td>1.50 (as citric)</td>
<td>1.50 (as citric)</td>
</tr>
<tr>
<td>Glucono-delta-lactone</td>
<td>3.50</td>
<td>11.1</td>
<td>0.81 (as citric)</td>
<td>1.50 (as citric)</td>
</tr>
</tbody>
</table>

1In juice expressed from sample.
more tart than the naturally fermented sauerkraut. A sweet aftertaste was not noted in this product, and the vegetable-like flavor and acid taste were clean, and very slightly citrus-like. Panelists considered this sample to be the most acceptable of the acidified products evaluated, but again its characteristics did not simulate those of natural sauerkraut. Apparently the processes involved in the disappearance of cabbage flavor compounds and development of the characteristic fermentation flavor compounds of sauerkraut are retarded or inhibited in directly-acidified cabbage preparation.

Samples of each of the acidified products were also cooked, but this did not cause liberation of sauerkraut tastes or odors. Further, even when samples which were acidified with acetic and lactic acid were refrigerated for 6 weeks, they did not develop sauerkraut tastes or odors.

The texture of the directly acidified products was very crisp, and resembled that of freshly-shredded cabbage. These samples appeared much firmer and crisper than the naturally-fermented sauerkraut, but the textures of both were similar after cooking. Blanching shredded cabbage before acidification with citric acid and subsequent equilibration resulted in a product with a texture similar to that of naturally-fermented sauerkraut before cooking. However, the texture of the blanched citric acid-acidified sample varied according to location in the equilibration container. The upper layers were softer than the lower layers. Blanching times of about 3 min appeared adequate to impart the sauerkraut-type of texture. After 3 min in the steam tunnel (96 C), centers of samples were 42 C, and the surfaces were 85 C.

Freezing the samples (-18 or -40 C) also resulted in a softer, more sauerkraut-like texture. However, both freezing treatments that were used increased the softness of the products excessively, and were not considered desirable.

In the second phase of the investigation, citric acid-acidified cabbage was prepared as described for the initial studies. This product was evaluated by sensory panels, and the results are in Table 3. It can be seen that the panelists could not detect a difference in the intensity of acidity or sweetness between the natural sauerkraut and the citric acid-acidified cabbage. Statistically significant differences were observed, however, for flavor type, texture, and preference. Absolute values of mean scores for flavor type showed that citric acid-acidified cabbage had a reasonably pronounced, green vegetable-like flavor, while the natural sauerkraut exhibited much less of this flavor. The softer texture of the natural sauerkraut could be attributed to canning as well as fermentation effects. Served chilled and without additional preparation, natural sauerkraut was preferred significantly over the citric acid-acidified cabbage. The lower preference for the direct acidified product may be partially attributed to panelists being unfamiliar with its distinctly different properties. When the two sauerkraut products were cooked with frankfurters, much less differentiation in preference was observed (Table 3), although the absolute preference score for the natural sauerkraut was still slightly higher.

It can be seen also that the other attributes were scored quite similarly for the two products, and support the preference data. It can be reasoned, therefore, that frankfurter flavor components as well as textural and flavor effects of heating resulted in development of

TABLE 3. Descriptive panel evaluation of citric acid-acidified and natural sauerkraut served chilled, and cooked with frankfurters.

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Acid flavor</th>
<th>Sweetness</th>
<th>Type flavor</th>
<th>Texture</th>
<th>Overall preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Served chilled</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural sauerkraut</td>
<td>4.76a</td>
<td>3.20a</td>
<td>3.40a</td>
<td>3.85a</td>
<td>4.08a</td>
</tr>
<tr>
<td>Citric-acidified sauerkraut</td>
<td>4.83a</td>
<td>3.10a</td>
<td>4.61b</td>
<td>5.59b</td>
<td>3.09b</td>
</tr>
<tr>
<td>STATISTICAL ANALYSIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-Value</td>
<td>ns7</td>
<td>ns</td>
<td>s</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td></td>
<td></td>
<td>0.75</td>
<td>0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>Served w/frankfurters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural sauerkraut</td>
<td>4.63a</td>
<td>3.25a</td>
<td>3.03a</td>
<td>3.36a</td>
<td>4.21a</td>
</tr>
<tr>
<td>Citric-acidified sauerkraut</td>
<td>4.37a</td>
<td>3.60a</td>
<td>3.53a</td>
<td>3.47a</td>
<td>3.82a</td>
</tr>
<tr>
<td>STATISTICAL ANALYSIS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-Value</td>
<td>ns7</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td></td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

1Scale: 1 = Extremely flat, bland; 7 = Extremely pronounced.
2Scale: 1 = Extremely lacking sweetness; 7 = Extremely sweet.
3Scale: 1 = Lacks green vegetable character; 7 = Extremely green, vegetable-like.
4Scale: 1 = Extremely soft, soggy; 7 = Extremely crisp, firm.
5Scale: 1 = Dislike extremely; 7 = Like extremely.
s = significant; ns = not significant; N = 24.
a,bMean scores for a given panel session in the same column with the same superscript are not significantly different at the five percent level.
similarities between the directly acidified and the natural products in the frankfurter combination item. Voluntary comments given by panelists in these two panel evaluations indicated that some panelists specifically liked the more crisp texture and less pronounced sulfury flavor of the citric-acid-acidified cabbage. Still, others found the naturally-fermented, traditional sauerkraut the much-preferred product.

Since the characteristic flavor of naturally-fermented sauerkraut was absent in the citric acid-acidified cabbage, addition of natural sauerkraut flavor via juice from a normal fermentation was investigated. Data in Table 4 show that the natural sauerkraut was preferred, but addition of natural sauerkraut juice improved the absolute preference score for the citric acid-acidified product although the increase was not statistically significant. As with earlier evaluations of chilled sauerkraut samples, acidity and sweetness mean scores were similar in products from each preparation method. Mean scores for the flavor-type attribute clearly show that addition of sauerkraut juice to the citric acid-acidified sample altered the overall flavor by lessening the green, vegetable-like flavor. Voluntary comments by panelists indicated also that some of the characteristics of natural sauerkraut flavor were imparted to the citric-acid-acidified product by the juice.

Additions of the sauerkraut juice to directly-acidified cabbage would be helpful to alleviate disposal problems for this material as well as to provide a natural flavoring source.

Of the acids evaluated citric acid provided the most desirable acidulant properties for the acidification of cabbage, and even though food-grade, pure citric acid was used, slightly citrus fruit-like flavors resulted. Further studies using lemon juice as a naturally-derived acidulent were carried out, and the resulting product was scored similar to citric acid-acidified cabbage in all aspects evaluated. These results indicated that lemon juice could be used for acidifying cabbage in direct-acid processing. However, some panelists commented that a bitter flavor was noted in the lemon juice-acidified sample, and this was attributed to bitter flavor components from the peel that were present in the lemon juice used. Avoidance of this flavor character probably would improve the quality of lemon juice-acidified cabbage.

Citric acid-acidified cabbage was included as an ingredient in a three-bean salad, and the results of the panel evaluation of this item are in Table 5. It can be seen that the citric acid-acidified samples received a higher absolute score, but it was not statistically preferred over the natural sauerkraut. Since the acidity

| TABLE 4. Descriptive panel evaluation of citric acid-acidified cabbage, citric-acid-acidified cabbage with natural sauerkraut juice, and natural sauerkraut. |
|---------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Sample description                                           | Flavor          | Sweetness       | Type flavor     | Texture         |
| Served chilled                                               | Descriptive scales 1 |
| Natural sauerkraut                                          | 4.96 a          | 3.33 a          | 2.62 a          | 3.45 a          | 4.37 a          |
| Citric acid-acidified sauerkraut                            | 4.93 a          | 3.26 a          | 4.73 b          | 5.62 b          | 3.05 b          |
| Citric acid-acidified sauerkraut with natural kraut juice   | 5.02 a          | 3.14 a          | 4.06 c          | 5.42 b          | 3.50 b          |

STATISTICAL ANALYSIS

<table>
<thead>
<tr>
<th>F-Value</th>
<th>LSD (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

| ns2 | ns | 0.46 | 0.29 | 0.47 |

1Same as listed in Table 3.
2ns = significant; ns = not significant; N = 29.
3Mean scores in the same column with the same superscript are not significantly different at the five percent level.

| TABLE 5. Descriptive panel evaluation of citric acid-acidified cabbage and natural sauerkraut served in a three-bean salad. |
|---------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Sample description                                           | Acid flavor     | Sweetness       | Type flavor     | Texture         |
| Three-bean salad                                            | Descriptive scales 1 |
| Natural sauerkraut                                          | 3.90 a          | 4.10 a          | 3.51 a          | 3.65 a          | 4.07 a          |
| Citric-acid-acidified sauerkraut                            | 3.68 a          | 4.27 a          | 5.03 b          | 5.69 b          | 4.43 a          |

STATISTICAL ANALYSIS

<table>
<thead>
<tr>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ns2</td>
</tr>
</tbody>
</table>

| ns | ns | 0.66 | 0.38 | ns |

1Same as listed in Table 3.
2ns = significant; ns = not significant; N = 29.
3Mean scores in the same column with the same superscript are not significantly different at the five percent level.
and sweetness attributes were scored similarly, it can be concluded that flavor-type and texture played roles in overall preference indications. The green, vegetable-like flavor and crispness of the citric acid-acidified cabbage were compatible with the salad, and some voluntary panelist comments indicated an association of the salad with coleslaw rather than sauerkraut salad. Apparently the polyphenolase enzyme system of the cabbage used in this study was effectively inhibited by the low pH and chelating effects of citric acid. All of the directly-acidified sauerkraut-like products appeared off-white in color, and did not exhibit noticeably green coloration due to the presence of chlorophyll. Non-enzymatic browning discolorations, sometimes encountered as a defect in canned naturally processed sauerkraut, were also not observed in any of the directly acidified products. However, this probably reflects an absence of heat-initiated reactions during the processing of the directly-acidified items.

In summary, these studies have shown that direct-acidification of cabbage gave sauerkraut-like products that showed potential use in some food applications. Direct-acidification products did not exhibit the typical, pronounced sauerkraut flavor and aroma, and were characterized by more crisp textures than canned naturally-fermented sauerkraut. These attributes could be used advantageously in development of additional acidified cabbage products, especially salad items. However, further testing of consumer reactions and evaluation of shelf stability would be appropriate before the extension of commercial production of directly-acidified cabbage products.

ACKNOWLEDGMENTS

The authors thank J. H. von Elbe and S. L. Karow for suggestions and assistance in the conduct of this project.

REFERENCES

Proteolytic Activity in Ultra-Pasteurized, Aseptically Packaged Whipping Cream


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ABSTRACT

The proteolytic activity in three commercial ultra-pasteurized, aseptically packaged samples of cream that developed bitter flavor was studied. Samples were analyzed for microorganisms and rate of proteolysis as affected by reaction temperature and pH. The temperature effect was determined at 20, 30, 37, 45, and 50 C. Samples A and B had an optimum temperature of 37 C for proteolytic activity while Sample C had its optimum between 30 and 37 C. Sample B had increased activity at pH 7.0, 8.0 and 9.0. Sample A had increased activity between pH 7.0 and 7.5 while Sample C exhibited greatest activity at pH 8.0. The rate of proteolysis increased with incubation time.

Because of shelf-life advantages, use of ultra-pasteurization and aseptic packaging techniques for cream product processing has increased. However, bitter flavor development has been a recurring problem. Extremely low microbial growth would indicate that bitter flavor development is related to heat stable proteolytic enzymes produced by bacteria before processing.

Proteolytic enzyme production by bacteria normally associated with milk has been investigated. In 1973 Juffs (4) examined bulk milk sources for soluble tyrosine values and microbial populations. Soluble tyrosine and total bacterial counts were significantly correlated but he found no relationship between soluble tyrosine and psychrotrophic or proteolytic psychrotrophic counts. In a later study (5) he stated that although psychrotrophic bacteria were important in production of proteolytic enzymes in milk, thermuduric bacteria produced a significant amount of protease. Mayerhofer et al. (6) studied a heat stable protease produced by Pseudomonas fluorescens P26. This enzyme required 15 h at 62.8 and 9 min at 121 C for complete inactivation. Milk, whey, and casein had a protective action against heat denaturation of this enzyme. As little as 0.2 unit of the enzyme caused bitter flavor development in milk within 30 days at 4 C.

White and Marshall (8) reported a definite reduction in the shelf-life and quality of cottage cheese and Cheddar cheese when products were made from milk inoculated with P. fluorescens P26 or the heat stable protease extracted from the bacteria. Adams et al. (1) found that nine of ten Pseudomonas species isolated from raw milk were capable of producing proteases which retained greater than 70% of their original activity after exposure to 149 C for 10 min.

The objectives of this study were (a) to investigate the proteolytic enzyme system in commercial cream samples that had developed bitter flavor during storage without microbial growth and (b) to determine if a commonly used method to study proteolysis in dairy products was sensitive enough to serve as a rapid screening test for cream upon receipt at the processing plant.

MATERIALS AND METHODS

Samples
Cream samples were obtained from three different commercial cream shipments. All samples had been sterilized, aseptically packaged and developed bitter flavor during storage. Each sample was plated according to Standard Methods (2). No growth was observed for any of the samples. Bitterness was then confirmed by sensory evaluation. The cream was separated by centrifugation at 1000 x g for 30 min. The resulting skim milk was used as the enzyme source. Sodium azide was added to a concentration of 0.02% to prevent bacterial growth.

Enzyme assay
Substrate for proteolytic activity determinations was a 2.5% solution of vitamin-free casein in 0.02 M phosphate buffer. The substrate was adjusted to the desired pH and made 0.02% in sodium azide.

The reaction mixture consisted of 5 ml of substrate and 2 ml of enzyme source. Following incubation, activity was terminated by addition of 10 ml of 0.72 N trichloroacetic acid (TCA). Controls were prepared by immediately mixing the substrate and enzyme with TCA. Release of TCA soluble tyrosine was determined according to Hull (3). Enzyme activity was reported as change in absorbance. Data presented are means of replicate trials.

RESULTS AND DISCUSSION

Figure 1 shows the proteolytic activity of each sample at pH 6.0 through 9.0 after incubation for 48 h at 37 C. Each sample appeared to have a distinct proteolytic
enzyme system. Samples A and C had their greatest activity at pH 7.5 and 8.0, respectively. Sample B apparently contained more than one proteolytic enzyme since there were enzyme activity peaks at pH 7.0, 8.0, and 9.0. If the proteolytic enzymes in these samples were from the same source all the samples should have had the same enzyme activity profile. The activity at pH 9.0 might have been the result of peptidase activity since many peptidases exhibit maximum activity in this pH range (7).

The effect of temperature on proteolytic activity at pH 7.5 is shown in Fig. 2. Sample B had the best defined optimum temperature and was most active at 37°C. If Sample B did contain more than one proteolytic enzyme as indicated by the pH effect, these enzymes were most active at 37°C. Sample A was active over the temperature range studied and only slightly affected by temperature. A slight increase in activity was observed at 37°C. Sample C had its greatest proteolytic activity between 30 and 37°C. The different optimum temperatures for the samples support the observation that each sample probably had unique proteolytic enzyme systems. Adams et al. (1) reported maximum activity for a partially purified heat stable protease at 45°C. Sample A was the only sample in this study that had significantly activity at 45°C.

To determine if the Hull (3) procedure was sensitive enough to rapidly detect proteolytic activity in cream samples so cream could be inspected before processing, samples were analyzed over 48 h at pH 7.5 and 37°C. Proteolytic activity was determined in each sample after 6, 12, 24, 36, and 48 h of incubation. All samples had similar response curves (Fig. 3). Very little proteolytic activity could be detected within 12 h but after 24 and 48 h activity was easily detected. The rate of proteolytic activity increased with time. This effect could be associated with a complex substrate such as casin. Initial activity might be slow but as the casein is progressively hydrolyzed the peptides could become more available to the enzymes. There were visible differences in the types of proteolytic activity. Sample C caused a fine granular precipitate in the reaction mixture while Sample B tended to digest the casein. Regardless of the cause of this effect the Hull procedure does not appear to be sensitive enough to rapidly detect low levels of proteolytic activity.

It seems possible that with a different substrate and assay procedure a test could be developed that would be capable of detecting low levels of proteolytic activity.

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preincubation of raw and laboratory pasteurized bulk milk samples
on tyrosine value and its relationship with bacterial populations. J.
Dairy Res. 40:383.
Characterization of a heat stable protease of Pseudomonas

location and characteristics of peptidase enzymes in lactic
streptococci. J. Dairy Sci. 60:710.
diary products by a heat stable protease from Pseudomonas

3-A Sanitary Standards Committees
Report of the Committee On Sanitary Procedures

Two regular meetings of 3-A were held since the last report.

3-A MEETINGS: 1. Hotel Savery, Des Moines, Iowa October
4, 5, 6, 1977.

Attendance and participation of all CSP members was commendable. Those unable to attend were prevented from being participants because of circumstances beyond their control.

Both meetings were responsive to the needs and consideration for updating and amending documents to make them sensitive to needs and requirements of present technology.


Members responding but unable to attend were: Joseph Stanley Karsh, Richard M. Parry, P. J. Benedetti.

Members present at the May 1978 meeting were: Peter J. Benedetti, Eddie R. Caraway, Dale R. Cooper, J. E. Edmondson, Joe W. Hall, Jr., Harold Johnson, Carl Kroppman, C. K. Luchterhand, Richard Webber, Dick B. Whitehead, Harold Irvin.

Members responding but unable to attend were: Anthony B. Bazzaro, Clinton Van Devender, W. K. Jordon, Joseph Stanley Karsh.

During the two sessions the following actions were taken involving CSP:
1. Seven (7) amendments were completed for Standards or Practices for signatures.
2. Comments were provided on three (3) Standards and three (3) Practices for return to task committees.
3. One (1) new Practice was completed for signatures.
4. Tentative revisions of two (2) Standards were studied and returned to task committees for action.
5. At the May 1978 meeting a "Report of DFISA Task Committee on Metric Practice" was received and discussed briefly—it will be considered further at our next regular meeting.

The items of "Old Business" and "New Business" are of great interest at this time.
Future meeting places are also included.

Report submitted by:
Dick B. Whitehead, R. S., Chairman Committee on Sanitary Procedures Consultant: Health and Safety 304 Forest Point Drive Brandon, Mississippi 39042 (601) 939-0397

Old Business
At the opening of the 3A meeting on May 2, the Chairman read a statement that reaffirmed 3A policy with regard to the attendance of manufacturer's consultants. The statement is included as part of these minutes, as follows:
The continued presence of specialized consultants to members of a DFISA Task Committee is cause for reaffirmation of ground rules on consultants established by the 3-A Committee many years ago. It is believed there are at least 2 consultants at the present meeting and there may be more. They are welcome at the open sessions. Letters of introduction have been a routine requirement in the past. It is the intent of the Steering Committee to continue this policy.
Participation by consultants who are not regular employees of Task Committee member companies is rather narrowly limited. Consultants are intended to speak on subjects confined to product lines of the client's manufacture. Consultants are not to be regarded as free agents. Their cooperation will be appreciated.

New Business
Two members of the DFISA Technical Committee proposed new tentative amendments to the published dryer practice, number 607-00. The first concerned fillet welds, and proposed new wording for D.2.4. The second, concerned special criteria for process air under 240F. These two proposals were discussed with the SSS for information and verbiage was presented.
The Steering Committee, in a separate action, authorized the DFISA Task Committee to initiate a new series of amendments under the SOP, designated T-607-02, for the purpose of including the foregoing proposals in principle, and also to include the D.8 section that was deleted from 607-01 as reported in Section VIII herein.

Meeting Sites
The next meeting of the 3A Committees is scheduled for April 10, 11, 12, 1979 in St. Louis, Mo. A partial fall meeting that year may be scheduled concurrent with Food and Dairy Expo at McCormick Place, Chicago, depending on the status of the accrued unfinished business resulting from the St. Louis 3A meeting. Beginning with 1980, 3A meetings will be scheduled for April and October dates in the even-numbered years, now that Food and Dairy Expo has switched to an odd-year schedule.

COMMITTEE ON SANITARY PROCEDURES IAMPES 1978

Whitehead, Dick B., Chairman; Consultant, Health and Safety, Brandon, Mississippi
Jordon, Dr. W. K., Vice Chairman; Cornell University, Ithaca, New York
Irvin, Harold R., Vice Chairman; Omaha-Douglas County Health Department, Omaha, Nebraska
Luchterhand, C. K., Division of Health, Madison, Wisconsin
Van Devender, Clinton, Mississippi State Board of Health, Jackson, Mississippi
Webber, Richard W., Standardization Branch, Poultry & Dairy Quality Division, FQS, USDA

Karsh, Joseph Stanley, Allegheny County Health Department, Pittsburgh, Pennsylvania
Benedetti, P. J., California Department of Food and Agriculture, Oakland, California
Cooper, Dale R., ManchesteR Milk Control Unit, Manchester, Iowa
Hall, Joe W., South Carolina Department of Health and Environmental Control, Columbia, South Carolina
Edmondson, Joe E., University of Missouri, Food Science and Nutrition, Columbia, Missouri
Kroppman, Carl, Florida Department of Agriculture and Consumer Services, Tallahassee, Florida
Bizzaro, Anthony B., Pennsylvania Department of Agriculture, Harrisburg, Pennsylvania
Johnson, Harold, Minnesota Department of Agriculture, Saint Paul, Minnesota
Caraway, Eddie R., Texas Department of Health Resources, Austin, Texas
Parry, Richard M., DVM, Connecticut Department of Agriculture (Retired), New London, Connecticut

PROTEOLYTIC ACTIVITY IN CREAM 45

location and characteristics of peptidase enzymes in lactic
streptococci. J. Dairy Sci. 60:710.
diary products by a heat stable protease from Pseudomonas

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PROTEOLYTIC ACTIVITY IN CREAM 45

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diary products by a heat stable protease from Pseudomonas
Bacteriological Survey of Frozen Mexican-style Foods Produced at Establishments Under Federal Inspection

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Beltsville, Maryland 20705

(Received for publication April 17, 1978)

ABSTRACT

During visits to 20 federally inspected establishments producing frozen Mexican-style foods (burritos, taco rolls, and tacos), 477 production line samples and 643 finished product units were collected for bacteriological analyses. The 46 sets of finished product (10 frozen Mexican-style foods (burritos, taco rolls, and tacos), 477 of these 46 sets had three or fewer coliform-positive units. Two or fewer aerobic plate count of fewer than negative for units/set) produced under good manufacturing practices had an levels in burritos, taco rolls and tacos during preparation and as packaged and frozen for shipment from establishments, with a mechanical dispenser. For either manually (with a spoon or scoop) or, as in most tortilla is folded once over the filling. By weight, each burritos and taco rolls, the tortilla is hand wrapped around the filling in a cylindrical shape. For tacos, the product is about always beef), water, beans and/or potatoes, tomato puree, textured vegetable protein, oat meal or flour, onion, and spices (salt, chili, cumin, pepper, garlic). There were some formulation variations among plants, but all fillings were very viscous with a pH range of 6.4.

The finished products, packed in retail or institutional sizes, are frozen before shipment.

MATERIALS AND METHODS

Sampling

At intervals from January 1971 to December 1976, samples were collected from 20 firms producing burritos, taco rolls, or tacos; most firms were visited and sampled more than once. Located on the West Coast and in the Southwest, the firms represent most of federally inspected establishments producing these products. A total of 477 production line samples and 643 finished product units were collected and analyzed. A group of samples per visit included samples of the ingredients, samples at each stage of production, a set of finished product units related to the production line samples, and a set of finished product units produced before the visit. Each unit was a single finished product and, nearly always, 10 units per set were collected. Samples were frozen promptly and shipped under dry ice to the laboratory.

During the visits to the firms, manufacturing practices were evaluated as “good” or “marginal” based on the food handlers' personal hygiene and the cleanliness of food contact surfaces before use, and the time and temperature the cooked components had been exposed to before use and the finished products had been exposed to before freezing.

Laboratory methods

Methods used for aerobic plate counts (APC), coliforms, Escherichia coli, Staphylococcus aureus, and salmonellae have been described (7).

Because of the uneven distribution of filling within each tortilla, an entire finished product unit, rather than a 50-g portion, was examined. A taco roll or taco was weighed and blended 2 min with the appropriate weight of diluent to attain the 1:10 dilution. A burrito was weighed and blended for 1 min with an equal weight of diluent and 100 g of this 1:1 dilution was blended 1 min with 400 g of diluent to attain the 1:10 dilution. Accordingly, the arithmetic average and geometric mean of the APC's of the units within a set were nearly always the same.

RESULTS AND DISCUSSION

At the time of initial sample collection, nine firms operated under marginal manufacturing practices (Table 1). In eight of the firms, the slow chilling rate of the viscous filling or the packaged finished product, or both, resulted in high bacterial counts. It had been a common practice to put the freshly cooked, hot filling into plastic lugs to a depth of 15-20 cm and place the lugs in coolers for a minimum of 24 h (in some cases, 4 days) before use. All samples of freshly cooked filling had, as expected, very low APC's. However, many samples of filling collected just before use had APC's ranging from 7 \times 10^4 to 1 \times 10^8. Each firm corrected this situation by adding filling to the lugs to a depth of only 8-10 cm and using the filling within 24 h; or by installing water-jacketed tanks with agitators to cool the filling below 25 C for use.
the same day. Another common practice had been the stacking of boxes of finished product (fabricated with warm filling) on a rack that, when filled, was wheeled into a freezer. Invariably, finished product units frozen promptly under dry ice for shipment to the laboratory had significantly lower APC's than the units frozen by this plant procedure. Placing boxes on the rack in a single layer reduced the product's insulation and reduced the time to fill the rack. This simple solution resulted in lower bacteriological counts. Other investigators have reported in more detail on the effect of the rate and method of cooling on foodstuffs (1-3, 6).

Firm K (Table 1) held filling in tubs at a depth of 20 cm for 4 days in a 10-C cooler. In addition, the food contact surfaces had not been properly cleaned and sanitized (the conveyor belt had a sour odor), and personal hygiene was poor. This firm ceased operations soon after the visit.

During a visit to Firm L (Table 1), finished product units frozen promptly under dry ice had high bacterial counts, although samples of the filling and tortillas soon after the visit were negative for E. coli. The finished products collected during subsequent visits had much lower bacterial counts.

On one occasion, a set of finished products collected in Firm P (Table 1) had a high incidence of E. coli. The production line samples implicated the pump dispenser system at the fabrication line as the contributing factor, and suggested that the system had not been properly cleaned and sanitized before use. Samples of filling at the entry of the system were negative for E. coli, but samples at the discharge of the system contained 100 E. coli/g.

The dominant microorganisms isolated from 26 finished product units with APC's greater than 1 x 10^6/g were streptococci, 95% of which were enterococci. Surprisingly, all these units were negative for gas-forming anaerobes in 0.1-g portions.

Table 2 presents the bacterial content of Mexican-style foods produced under good manufacturing practices and includes those firms that had operated under marginal manufacturing practices before corrections.

In all, sanitary conditions in the firms listed in Table 2 were good. Food contact surfaces were treated with a sanitizing agent after cleaning, filling-dispenser systems were disassembled for cleaning and sanitizing, hand sanitizing solutions were used by employees, and cooked ingredients and finished products were chilled promptly. Thus, despite the extensive manual handling of the product, close attention to sanitary practices resulted in a low incidence of indicator microorganisms (Table 2). All units were negative for E. coli and salmonellae; only 7

**TABLE 1. Bacterial content of Mexican-style foods produced under marginal manufacturing practices.**

<table>
<thead>
<tr>
<th>Firm</th>
<th>No. of sets</th>
<th>No. of units ( \times 10^3 )</th>
<th>No. of units with ( \times 10^3 )</th>
<th>Range of APC's/g sets (geom. means)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>( 3 \times 10^3 )</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>( 6 \times 10^4 )</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>( 10 \times 10^4 )</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>( 10 \times 10^2 )</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^1 )</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^0 )</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^3 )</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^4 )</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^5 )</td>
</tr>
<tr>
<td>J</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^6 )</td>
</tr>
<tr>
<td>K</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^7 )</td>
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<tr>
<td>L</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^8 )</td>
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<tr>
<td>M</td>
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<td>4</td>
<td>2</td>
<td>( 10 \times 10^9 )</td>
</tr>
<tr>
<td>N</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^{10} )</td>
</tr>
<tr>
<td>O</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^{11} )</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^{12} )</td>
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<tr>
<td>Q</td>
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<td>4</td>
<td>2</td>
<td>( 10 \times 10^{13} )</td>
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<td>( 10 \times 10^{14} )</td>
</tr>
<tr>
<td>S</td>
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<td>4</td>
<td>2</td>
<td>( 10 \times 10^{15} )</td>
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<tr>
<td>T</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>( 10 \times 10^{16} )</td>
</tr>
</tbody>
</table>

\( a \) All units were salmonellae-negative in 25 g portions.

**TABLE 2. Bacterial content of Mexican-style foods produced under good manufacturing practices.**

<table>
<thead>
<tr>
<th>Firm</th>
<th>No. of sets</th>
<th>No. of units ( \times 10^3 )</th>
<th>No. of units with ( \times 10^3 )</th>
<th>Range of APC's/g sets (geom. means)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 1 \times 10^3 )</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 2 \times 10^3 )</td>
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<tr>
<td>C</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 3 \times 10^3 )</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 4 \times 10^3 )</td>
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<tr>
<td>E</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 5 \times 10^3 )</td>
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<tr>
<td>F</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 6 \times 10^3 )</td>
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<tr>
<td>G</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 7 \times 10^3 )</td>
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<td>H</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 8 \times 10^3 )</td>
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<tr>
<td>I</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 9 \times 10^3 )</td>
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<tr>
<td>J</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 10 \times 10^3 )</td>
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<td>K</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 11 \times 10^3 )</td>
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<td>L</td>
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<td>0</td>
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<td>M</td>
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<td>N</td>
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<td>0</td>
<td>( 14 \times 10^3 )</td>
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<tr>
<td>O</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 15 \times 10^3 )</td>
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<td>P</td>
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<td>0</td>
<td>0</td>
<td>( 16 \times 10^3 )</td>
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<td>Q</td>
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<td>0</td>
<td>( 17 \times 10^3 )</td>
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<td>R</td>
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<td>( 18 \times 10^3 )</td>
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<td>S</td>
<td>1</td>
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<td>0</td>
<td>( 19 \times 10^3 )</td>
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<tr>
<td>T</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( 20 \times 10^3 )</td>
</tr>
</tbody>
</table>

\( a \) All units were salmonellae-negative in 25-g portions.
(1.5%) of the 460 units were \textit{S. aureus}-positive (all in 0.1 g portions); and 78 (17%) were coliform-positive, with only three containing as many as 1,000 coliforms/g.

As indicated previously, the major problem in the firms that operated under marginal manufacturing practices was the slow cooling rate of the filling or the finished product. Figure 1, by comparing the APC’s of units produced under good and marginal practices, shows the effect of a slow cooling rate on these foods.

This survey shows that at plant level and by the laboratory methods employed, 46 sets of the finished product units of burritos, taco rolls, or tacos (10 units/set) produced under good manufacturing practices had an APC of fewer than 50,000/g (geometric mean), and two or fewer \textit{S. aureus}-positive units. Most sets (78%) had three or fewer coliform-positive units. All units were negative for \textit{E. coli} and salmonellae.

During this study, groups of enchilada samples were collected from three firms, each operating with good manufacturing practices. Enchiladas, although prepared like burritos, were not included in this survey, because they are garnished with raw cheese as a final processing step. Before garnishing, the enchilada samples had very low APC’s ranging from 300 to 3,000/g. The high quality cheeses had APC’s ranging from $3 \times 10^6$ to $2 \times 10^7$/g. The high quality cheeses had APC’s ranging from $3 \times 10^4$ to $3 \times 10^5$/g. These findings are presented here to demonstrate the pitfalls of objective sampling, particularly at retail level. As reported in earlier surveys (4,5), a knowledge of the various food processing procedures and the examination of line samples are necessary to properly interpret the results of bacteriological analyses of many foods.

**ACKNOWLEDGMENT**

We thank the Microbiology Staff for their professional assistance. We are grateful for the full cooperation of personnel of the Western and Southwestern Regions, USDA Meat and Poultry Inspection Program.

**REFERENCES**

Behavior of *Clostridium botulinum* in Vacuum-Packed Fresh Celery

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University of Wisconsin-Madison
Madison, Wisconsin 53706

(Received for publication May 8, 1978)

ABSTRACT

Sliced fresh celery pieces were placed in barrier pouches (1 mm nylon/2.5 mm polyethylene) and then inoculated with types A, B, and E *Clostridium botulinum* spores (10^7/454 g). Pouches were sealed after evacuating, flushing with nitrogen, or without alteration, and were then incubated at 7 or 21 C for up to 8 weeks. None of these inoculated samples yielded *botulinum* toxin. Fortification of vacuum-packaged celery with nutrient broth resulted in toxin production at 21 C after 8 weeks. All results indicated that fresh celery held anaerobically under simulated commercial refrigerated storage conditions will not support growth and toxin production by *C. botulinum* because of nutrient insufficiency or other inhibition factors.

There is a growing market for ready-to-use fresh vegetables packaged in plastic bags. Although the bags are usually ventilated with holes or not sealed at the top, recent work has indicated that the storage life of shredded lettuce and broccoli is improved by sealing the bags. Further, Wu (9) has recommended packaging fresh broccoli in polyethylene under vacuum.

Respiration of the product within a semipermeable package could produce an anaerobic condition which would be favorable for growth of *Clostridium botulinum*. Sugiyama and Yang (6) reported oxygen levels of less than 2% resulted in production of *botulinum* toxin in inoculated fresh mushrooms wrapped in polyvinylchloride (PVC) film and stored at 20 C. To prevent production of botulinum toxin workers have recommended one (7) or two (4) 1/8-inch ventilation holes in 1-lb. packages of PVC-wrapped fresh mushrooms.

Recent work has shown that fresh sliced celery can be stored for at least a week in polyethylene lined storage bins (3). Since celery benefits from low-oxygen storage (2), sealing the bins may be beneficial. This study determined the potential for toxin production by *C. botulinum* in anaerobically packaged, fresh sliced celery stored at 7 C.

MATERIALS AND METHODS

Mature, locally grown, fresh celery was washed; trimmed to exclude leaves, butt and heart portions; and cut into 3/4-in. slices. The slices were hydrocooled below 4 C and 1-lb. (454 g) lots of celery were placed in pouches constructed of nylon/polyethylene (1 mm/2.5 mm) supplied by Curwood, Inc., New London, Wisconsin. Gas transmission rates per 100 inches² (645 cm²) of film were 2-3 cm³ for oxygen and 100 cm³ for carbon dioxide per 24 h at 25 C (manufacturers specifications).

**Pouch preparation**

Twelve strains (five type A, five type B, and two type E) of *C. botulinum* were used in this study. Spores were harvested from individual cultures of each strain grown in a soil-enriched medium that is conducive to sporulation. A mixed-strain spore suspension containing 10^8 spores per ml was prepared in a phosphate-saline diluent. The spores were heat shocked at 80 C for 10 min before inoculating the celery.

**Pouch inoculation and incubation**

One ml of spore suspension was added to each pouch containing celery. Pouches were kneaded to distribute the inoculum and then sealed using a Multivac Mod. AG5 vacuum packaging machine (Koch Supplies, Inc., Kansas City, MO.). Three lots were prepared: (a) one under a vacuum with a pressure of 125 mm Hg; (b) another with an ambient pressure of nitrogen after evacuation and introduction of nitrogen gas; and (c) a lot which was sealed containing atmospheric gases at ambient pressure. In addition, a positive toxin producing control series was prepared for incubation at 21 C in which each pouch of celery received 3.0 ml of nutrient broth to assure suitable growth conditions for *C. botulinum*.

**Toxicity assay**

The procedure of Kautter and Lynt (5) was employed in this study. After 1, 2, 4, and 8 weeks of incubation, duplicate 25-g samples were removed from pouches and each was individually blended in a phosphate-gelatin buffer. Each preparation was trypsinized to enhance detection of type E toxin and then injected into Albino mice.

RESULTS AND DISCUSSION

No botulinial toxin was detected in any of the samples of sliced celery incubated at 7 C for up to 8 weeks. These negative results may have been aided by the low temperature of incubation which was not conducive to either germination of the spores or production of toxin.

The celery samples incubated at 21 C showed visible deterioration after 2 weeks, but neither the nutrient broth fortified nor the non-fortified celery samples contained botulinial toxin (Table 1). Tests with the non-fortified celery stored at 21 C were terminated after 2 weeks as the extensively deteriorated condition of the celery precluded any consideration of the celery as a food product at this point.
To demonstrate the viability of the botulinum cultures employed, incubation at 21 C of pouches of celery fortified with nutrient broth was continued to 8 weeks. At this time the celery samples in the vacuum-sealed pouches contained botulinal toxin. However, the nitrogen-flushed pouch gave a negative test result. The reason for the negative result is unknown, except that absolute anaerobiosis may not have been obtained, or possibly competitive inhibitory overgrowth by other organisms may have occurred.

The vacuum-packaged celery samples with nutrient broth fortification incubated at 21 C for 8 weeks showed definite \textit{C. botulinum} toxin formation. The test mice showed toxic symptoms approximately 20 h after injection and positive results were obtained with both trypsinized and non-trypsinized preparations. Mice protected with the coincident administration of type A and B antiserum did not evidence botulinal symptoms, whereas the control animals died. This indicated a limited production of either type A or B toxin in that sample. The slow rate of production of \textit{C. botulinum} toxin in the celery samples fortified with nutrient broth (8 weeks at 21 C) may have been a result of growth inhibition factors in the fresh celery or from competitive microbial activity in the medium.

In summary, results indicate that fresh celery stored under low oxygen conditions at 7 or 21 C is not a conducive medium for production of \textit{C. botulinum} toxin. This may be attributed to insufficient nutrients, adverse temperatures, or other inhibitory factors. Therefore, it can be concluded that fresh celery will not support \textit{C. botulinum} growth and toxin production when stored under commercial refrigeration even if anaerobic conditions are encountered.

**ACKNOWLEDGMENTS**

This work was supported in part by the College of Agriculture and Life Sciences, University of Wisconsin-Madison and by Leach Farms, Inc., Berlin, Wisconsin. The toxicity assays were conducted by Madison Bacteriology Laboratories, Inc., Madison, Wisconsin.

**REFERENCES**

Inoculation of Citric Acid-Fermenting Bacteria into Raw Milk in Farm Bulk Tanks

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(Received for publication April 20, 1978)

ABSTRACT

Cultures of Streptococcus lactis subsp. diacetylactis and Leuconostoc cremoris were added together in the amount of 0.5% to raw milk in a farm bulk tank. This treatment did not significantly reduce the psychrotrophic or coliform population as hypothesized; however, the shelf-life was extended on products made from this raw milk by an obvious fact is noted that this agent can already be added to milk the toxic agent (to the psychrotrophs) released by the citrate fermenters, the raw milk needs to be considered by state health departments and appropriate federal agencies. Since hydrogen peroxide is reported to be the agent which tend to permit the entry of psychrotrophs into the farm bulk tank. This treatment did not significantly reduce the average of psychrotrophic or coliform bacteria. Work in the laboratory and Babel's hypothesis quite effectively. Juffs and Babel (l) postulated that the killing action was not due to the lactic acid produced by the cultures but more likely due to hydrogen peroxide. These workers supported this hypothesis quite effectively.

Since psychrotrophic bacteria are present in almost all raw milk supplies, they constitute the major problem concerning shelf-life reduction in milk and milk products. Nearly all pasteurized milk held at 4°C or below will eventually develop off-flavors due to psychrotrophic growth. Thus research which can reduce the number of psychrotrophs present in raw milk takes on added significance. Admittedly, the best way of reducing or retarding psychrotrophic growth is by preventing their entry into the milk at the farm. While prevention is the best method and one which is technically feasible, it is not easily achieved. Improper washing and drying procedures and improperly cleaned and sanitized farm bulk tanks head the list of factors which tend to permit the entry of psychrotrophs into the raw milk.

Therefore, the primary purpose of this study was to investigate the feasibility of adding dairy cultures capable of fermenting citric acid to raw milk on a commercial basis. The effect of these added cultures on the normal microflora would be observed. Moreover, the effect, if any, addition of these cultures would have on the shelf-life of dairy products made from this raw milk would be noted. This research was deemed important since conditions at the farm level are often hard to duplicate in the laboratory thus making long-term feasibility studies difficult.

MATERIALS AND METHODS

Cultures

Commercial lyophilized cultures of citrate-fermenting bacteria (Streptococcus lactis subsp. diacetylactis - #188 and Leuconostoc cremoris - CAF) were obtained from a commercial source. The cells were grown in reconstituted (10% MSNF) milk that had been steamed for 1 h. Initial inoculation was 1% with subsequent incubation at 21°C for 16 h. Following two transfers, bulk cultures were prepared and held at 4°C until used.

Initial laboratory testing

Before starting the study involving farm milk, attempts were made to simulate what would occur in the bulk tank. The situation of everyday pickup or every-other-day pickup would need to be considered. The effect of warming the milk as would occur during a normal milking would need to be considered. This is important since no matter how good a refrigeration system, there will be at the very minimum localized warming. This will obviously vary from farm to farm.

Growth responses (total count, pH, and titratable acidity) were obtained for the two organisms, S. lactis subsp. diacetylactis and L. cremoris, both in skim milk and in raw whole milk. This was done in the laboratory before any fieldwork. Growth responses were measured at 4.4, 10, and 21°C. Finally, the responses were measured after 3 h at 4.4°C, 10 h at 10°C, again after 3 h at 4.4°C, 10 h at 10°C, etc. This was an attempt to predict the actual response of the cultures in the bulk tank. The temperature of the milk in the bulk tank at the University of Georgia Dairy Research Center rose to 10°C during milking but was back down to 4.4°C or less within 30 min postmilking; thus the conditions selected for the laboratory study were harsher than those the cultures would encounter during the actual experiment. The obvious reason for these safeguards was because S. lactis subsp. diacetylactis produces lactic acid.

Present address: Dean Foods Company, 1126 Kilburn Ave., Rockford, Illinois.
**Experimental procedure**

When assurance was obtained from the laboratory studies that the cultures would not coagulate the milk in the farm bulk tank, the main thrust of the study commenced. An outline of the experimental procedure is depicted in Fig. 1.

Cultures were added to approximately 0.5% by weight following the evening milking. The average weight of milk for the two milkings was 2165 kg (5500 lb.). The milk was picked up daily from the University Farm following the morning milking. Thus, the culture was added at 0.5% based on the estimated quantity of milk that would be present after two milkings.

Stage one represents the raw milk before treatment. The Standard Plate Count (SPC), coliform count (COLI), 10-day psychrotrophic count (PSY), pH, and titratable acidity (TA) were determined.

Stage two represents the raw milk immediately (5 min) following addition of culture. The same measurements as for stage one were taken. Stage three represents the same milk taken from the tank truck.

Stage four represents the same milk taken from the raw milk holding tank at the University processing plant. The pH and TA were not taken on the samples from any subsequent stage.

Stage five represents a 10-ml sample taken from the raw milk both before and after addition of starter. The sample was pasteurized in the laboratory at 63 C - 30 min — the SPC being determined both at 32 and 21 C (48-h incubation). Coliform and psychrotrophic counts were also determined.

Stage six represents the finished product (homogenized milk and chocolate milk) made from the same supply of raw milk. The samples were evaluated for flavor and bacterial estimates.

Stage seven represents a 10-ml sample taken again from the raw milk both before and after addition of culture. These samples were incubated raw at 7 C for 5 days after which bacterial estimates were made.

Stages eight and nine represent samples of finished product examined after refrigerated (4.4 C) storage for 7 and 10 days, respectively. The samples were tasted following plating.

Stage 10 represents the resulting shelf-life of the finished samples, i.e., days required before off-flavor development.

The procedure was repeated weekly for 9 weeks. Untreated samples were taken from six different loads of milk coming into the UGA processing plant (stage three). Untreated samples were taken to give a baseline and a picture of the natural microflora of the raw milk.

**RESULTS AND DISCUSSION**

The two strains of citrate fermenters grew adequately in reconstituted (10%) NFDM. Initially, there was some difficulty in obtaining sufficiently large numbers of *L. cremoris* (CAF). The amount of inoculum was increased to 6% with a resultant increase in numbers following incubation. The mean count of the CAF culture was $98 \times 10^6$ cfu/ml while the mean count for 188 was $1.96 \times 10^8$ cfu/ml. The two organisms grew quite well in both types of media, skim milk and raw milk.

With regard to pH, culture CAF did not lower the pH below 6.6 even after 36 h of alternating incubation temperatures between 4.4 C (3 h) and 10 C (10 h). With culture 188, however, the situation was a little more critical since the pH was reduced to 4.8 after the same 36 h. Thus, when the study was initiated, the amount of culture used to inoculate milk in the farm bulk tank was carefully monitored with 75% of the total starter being CAF and 25% made up of 188 (Table 1). With this combination, there was no appreciable rise in acidity when using everyday pickup from the farm. The mean pH after addition of the milk from the second milking was 6.75 with a mean TA of .18. Actually a higher percentage of CAF alone may eventually prove to be best, depending upon the degree of psychrotrophic reduction obtained. If culture 188 were solely used with less than

![Figure 1](image-url)
TABLE 1. Composition of dairy culture used to inoculate raw milk in farm bulk tank.

<table>
<thead>
<tr>
<th>Culture used</th>
<th>Total starter</th>
<th>Approximate No. cells¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuconostoc cremoris (CAF)</td>
<td>75</td>
<td>99 × 10¹⁰</td>
</tr>
<tr>
<td>Streptococcus lactis subsp. diacetylactis (188)</td>
<td>25</td>
<td>16 × 10¹¹</td>
</tr>
</tbody>
</table>

¹Represents total number of cells added of each bacterial strain.

In adequate refrigeration in the tank, the acidity could most assuredly increase to prohibitive levels.

In contrasting stages 1 and 2 (Fig. 1) which involve the raw milk before and after addition of culture, there was a highly significant (P < 0.01) difference between stages only in SPC values. This significance was expected since actively growing cells were added which readily showed up on plates incubated at 32 C for 48 h. Data in Table 2 show the actual difference.

TABLE 2. Mean differences between bacterial estimates¹ of raw milk before and after addition of citrate fermenting bacteria.

<table>
<thead>
<tr>
<th>Stage</th>
<th>SPC</th>
<th>PSY</th>
<th>COLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Before)</td>
<td>3.498³</td>
<td>2.0089</td>
<td>1.9864</td>
</tr>
<tr>
<td>2 (After)</td>
<td>5.5572</td>
<td>2.6734</td>
<td>1.9112</td>
</tr>
</tbody>
</table>

²All values are log means.
³P < 0.01.

In following the milk through the outline (Fig. 1), the milk is pumped into the tank (stage 3) where it is transported to the processing plant (stage 4). There were no significant differences between these two stages either in counts or in acidity (pH or TA). There were some highly significant (P < 0.01) differences among treatments (treated vs. non-treated), however. These differences are depicted in Table 3. The high psychrotrophic count in the treated samples indicates that a portion of the cultures are at least capable of some growth at 7 C. Gram staining of cells from plates made of starter psychrotrophic plate indicated a mixture of gram negative rods, gram positive rods, and gram positive cocci.

With regard to stage 5, which involved laboratory pasteurization of milk taken from stages 1 (before), 2 (after), and 3 (for non-treated samples only), there were no significant differences in SPC (log) values between temperatures of incubation (21 vs. 32 C) although the higher temperature (32 C) resulted in slightly higher counts (1.5476 at 32 C and 1.4384 at 21 C). Also, the treated milk had a slightly larger number of survivors than did the non-treated milk (1.5022 vs. 1.3893). Likewise, there were no significant differences for either psychrotrophs or coliforms when comparing the treated vs. non-treated samples.

In stage 7, raw milk samples were held for 5 days at 7 C to observe the unrestricted growth of microorganisms capable of growth at refrigeration temperatures. In the first comparison, stage 2 (after addition of culture) is contrasted with stage 3 (non-treated only) for SPC, PSY, and COLI. These data are in Table 4. No significant differences were noted.

TABLE 4. Comparison of log means of bacterial counts involving milk to which culture was added and a non-treated sample after holding raw milk at 7 C for 5 days.

<table>
<thead>
<tr>
<th>Test</th>
<th>Stage</th>
<th>Mean</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>2³</td>
<td>7.1279</td>
<td>N.S.</td>
</tr>
<tr>
<td>PSY</td>
<td>2³</td>
<td>6.7207</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>2³</td>
<td>6.4814</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

⁴Stage 2 = after addition of culture
⁵Stage 3 = non-treated milk.

In looking at only the treated milk before and after addition of culture, a somewhat clearer picture is obtained. These data (for stage 7) are in Table 5. The SPC shows a significantly higher number of bacteria present in the treated sample — an expected happening. There was a slight decrease in the number of coliforms in the treated samples but a slight increase in the number of psychrotrophs, an unexpected and undesirable event. This comparison is of particular importance since raw milk is being held for extended periods and the nature of the microflora of the raw milk needs to be examined.

The most important criterion comes, however, with stages 6, 8, and 9, i.e., the finished product (homogenized milk and chocolate milk) and the shelf-life of these products (stage 10). Initially, comparing stages 6 (day of packaging), 8 (after 7 days of storage at 7 C), and 9 (after 10 days of storage at 7 C) for homogenized milk, highly significant differences were obtained for SPC,

TABLE 5. Comparison of log means of bacterial counts involving treated milk both prior to addition of culture and after with both samples subsequently being held raw for 5 days at 7 C.

<table>
<thead>
<tr>
<th>Test</th>
<th>Stage</th>
<th>Mean</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>Before</td>
<td>6.0811</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>SPC</td>
<td>After</td>
<td>7.1279</td>
<td>N.S.</td>
</tr>
<tr>
<td>PSY</td>
<td>Before</td>
<td>6.0780</td>
<td>N.S.</td>
</tr>
<tr>
<td>PSY</td>
<td>After</td>
<td>6.3106</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>Before</td>
<td>3.0454</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>After</td>
<td>2.6628</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
The differences were among stages and not for treatment, i.e., treated vs. non-treated. The increase in mean psychrotrophic count closely paralleled that of the total aerobic, mesophilic count (SPC).

**TABLE 6. Differences of bacterial counts after five and seven days of refrigerated storage of pasteurized, homogenized milk made from milk treated with citrate fermenting bacteria.**

<table>
<thead>
<tr>
<th>Test</th>
<th>Stage</th>
<th>Mean(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>6(^1)</td>
<td>1.3947 a(^5)</td>
</tr>
<tr>
<td></td>
<td>8(^2)</td>
<td>3.3872 b</td>
</tr>
<tr>
<td></td>
<td>9(^3)</td>
<td>5.1183 c</td>
</tr>
<tr>
<td>PSY</td>
<td>6</td>
<td>0.4985 a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.6332 b</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4.4865 c</td>
</tr>
<tr>
<td>COLI</td>
<td>6</td>
<td>0.0000 a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.9084 b</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.0227 b</td>
</tr>
</tbody>
</table>

\(^1\)Stage 6 = Day of bottling.  
\(^2\)Stage 8 = After 5 days of storage at 7 C.  
\(^3\)Stage 9 = After 7 days of storage at 7 C.  
\(^4\)Log values  
\(^5\)Any means not followed by the same lower case letter differ significantly (P < 0.05).

When F tests were made on the chocolate milk, almost exact counts were obtained as had been obtained for the homogenized whole milk. Thus significant increases occurred among counts over refrigerated storage but no significant differences were noted for treated vs. non-treated samples.

Shelf-life values for both the homogenized milk and chocolate milk for treated (culture added) and non-treated samples are shown in Table 7. The shelf-life values indicate that the milk tended to last 1 day longer with rather than without the citrate fermenting bacteria added to the raw milk. While this is not a tremendous extension, it is a promising start and future research is needed to further extend this work.

**TABLE 7. Shelf-life values for homogenized whole milk and chocolate milk which either did or did not have added citrate fermenting bacteria added to the raw milk before processing.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Shelf-life (days - mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenized whole milk</td>
<td>Treated(^2)</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>Non-treated(^2)</td>
<td>15.3</td>
</tr>
<tr>
<td>Chocolate milk</td>
<td>Treated</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>Non-treated</td>
<td>12.4</td>
</tr>
</tbody>
</table>

\(^2\)Treated = Citrate fermenters added to raw milk.  
\(^2\)Non-treated = No bacteria added.

**REFERENCES**

Chemical Destruction of *Mycobacterium bovis* in Milk

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(Received for publication May 5, 1978)

ABSTRACT

The tuberculocidal activity of phenol and 1-Stroke Environ was tested using five *Mycobacterium bovis* strains added separately to five samples of untreated cows milk. The tuberculocidal activity of each disinfectant was significantly improved (P = .005) by increasing exposure temperature from 4 to 23 C or by increasing exposure time from 1 to 6 h or by increasing disinfectant concentrations two-fold.

Environ diluted 1:8 or phenol diluted 1:32 killed each of the five strains of *M. bovis* suspended in untreated milk (6 mg/ml) during a 6-h exposure at 23 C. Either disinfectant could be used to destroy *M. bovis* in unsalable milk from tuberculous cows scheduled for slaughter.

*Mycobacterium bovis* in milk from tuberculous cows was a major human health problem before pasteurization of milk and continues to be a threat to the health of animals (1). Disposal of milk from tuberculous cows was scheduled for slaughter presents an unusual decontamination problem today. Chemical treatment appears to be a feasible method for killing *M. bovis* in milk, thereby eliminating the hazard of bacterial contamination of the environment when unsalable milk is discarded.

Although the tuberculocidal activity of several disinfectants has been studied (3), there is currently no standard method for chemical destruction of *M. bovis* in milk. The purpose of this study was to evaluate two disinfectants, phenol and 1-Stroke Environ, for destroying *M. bovis* in milk. Phenol was chosen because it is a reference compound, readily available and proven effective as a tuberculocide (6). Environ was chosen because it is an approved USDA tuberculocide and was superior to other substituted phenolic tuberculocides tested in a preliminary probe.

MATERIALS AND METHODS

Disinfectants

Two disinfectants were used in the study: 1-Stroke Environ germicidal detergent (o-phenylphenol 10.0%, 0 benzyl-p-chlorophenol 8.5%, p-tertiary-amyphenol 2.0%) (Vestal Laboratories, St. Louis, Mo. 63110); and liquifed phenol, (Mallinckrodt Inc., St. Louis, Mo. 63160). Both are hazardous chemicals (4).

Cultures

*M. bovis* strains 878 (ATCC 19211), 1042, 1332, 1505, and 1606 were isolated in this laboratory from bovine tissue.
to determine whether or not the normal bacterial flora, measured in the negative milk controls, was killed by the routine test concentrations of each disinfectant.

Each M. bovis strain was reisolated on Herrold's medium from turbid growth in two separate tubes of DTA; one had survived phenol treatment and the other had survived Environ treatment. Isolated colonies on Herrold's medium were typed by routine biochemical methods (7).

Smears were prepared from approximately 20% of the final dilutions which produced 0.05 O.D. or greater. They were stained by the Ziehl-Neelsen method (7) to determine if acid-fast test cultures or non-acid-fast milk flora had produced the turbid growth.

**Statistical analysis**
The sign test (5) was used to determine the statistical significance of exposure temperature, exposure time, and disinfectant concentration on the tuberculocidal activity of Environ and phenol. An analysis was made of the number of times each variable caused an increase, a decrease, or no change in the tuberculocidal activity of each disinfectant.

**RESULTS AND DISCUSSION**
The tuberculocidal activity of both disinfectants increased with increased temperature, time, or disinfectant concentration (Tables 1, 2). Four comparisons were excluded due to contamination. In 63 of 116 trials (41 Environ, 22 phenol), the tuberculocidal activity was greater at 23 C than at 4 C. In 53 trials (19 Environ, 34 phenol) there was no difference in activity at the two temperatures. In 56 of 116 trials (38 Environ, 18 phenol) the tuberculocidal activity of the disinfectants was increased when the exposure time was extended from 1 to 6 h. There was no increased activity in 60 of the 116 trials (22 Environ, 38 phenol). Increased disinfectant concentrations caused increased tuberculocidal activity in 96 of 156 trials (63 Environ, 33 phenol). In 60 trials (17 Environ, 43 phenol), there was no difference in disinfectant activity between consecutive two-fold dilutions.

In the statistical analysis, the computed chi-square values all exceeded 15.0, indicating a highly significant effect of exposure temperature, exposure time, and disinfectant concentration on tuberculocidal activity (P = 0.005). These data are summarized in Table 2.

The highest dilution of phenol which was capable of killing the five M. bovis strains was 1:32 when exposure was made at 23 C for 1 or 6 h. When exposure was at 4 C for 6 h, the highest effective dilution was 1:16. Growth occurred in all dilutions of phenol when exposure was at 4 C for 1 h. The highest dilution of Environ which killed the five M. bovis cultures was 1:8 when exposure was made at 23 C for 6 h; 1:4 at 23 C for 1 h. Growth occurred in all dilutions of Environ when exposure was made at 4 C.

Although no precise comparisons of strain-related sensitivity to disinfectants were made, similarities between strains were evident in their rate of growth. In positive dilution control tests (no disinfectant), strain 878 first appeared at 11 days while the other four strains first appeared at 8 days. The earliest appearance time for strains treated with disinfectants was 8 days. Strain 878 did not appear until 29 days. The latest appearance time was 36 days. The variations in appearance time are shown in Table 1.

The opacity of the untreated milk caused a relatively high initial O.D. (average 1.2) in the 1:100 dilution. The turbidity increased rapidly to an average maximum O.D. of 1.7 in 24 h. The initial O.D. was 0 for all 1:10,000

---

**TABLE 1.** The effect of temperature, time and disinfectant concentration on appearance time of growth. Average of 5 M. bovis isolates.

<table>
<thead>
<tr>
<th>Temp (C)</th>
<th>Exp</th>
<th>Environ</th>
<th>Phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1:4</td>
<td>1:8</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>36*</td>
<td>24.4*</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>36*</td>
<td>36*</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>14.8</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11-22)</td>
<td>(8-15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34.6</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22-36*</td>
<td>(11-22)</td>
</tr>
</tbody>
</table>

*Exposure time in hours.

*Disinfectant dilution.

*First appearance of growth. Upper number = mean days. Numbers in parentheses = range of days.

*36* No growth during 36-day incubation period.

**TABLE 2.** Summary of effects of temperature, time and disinfectant concentration on tuberculocidal activity of Environ and phenol.

<table>
<thead>
<tr>
<th>Tuberculocidal activity</th>
<th>Exposure temperature (4 to 23 C)</th>
<th>Exposure time (1 h to 6 h)</th>
<th>Disinfectant concentration (two-fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>41</td>
<td>23*</td>
<td>38</td>
</tr>
<tr>
<td>No change</td>
<td>19</td>
<td>34</td>
<td>22</td>
</tr>
<tr>
<td>Decreased</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chi-square</td>
<td>39.0</td>
<td>20.0</td>
<td>36.0</td>
</tr>
</tbody>
</table>

*Number of trials representing Environ.

*Number of trials representing phenol: four trials excluded due to contamination.

*Computed chi-square, comparing number of increases vs. number of decreases in tuberculocidal activity.
dilutions of untreated milk and then increased rapidly to an average maximum O.D. of 0.65 in 4 to 8 days of incubation. No growth occurred in any of the chemically treated milk controls indicating a complete kill of the normal milk bacterial flora.

Each _M. bovis_ strain was reisolated from DTA following separate exposure to phenol and Environ. Biochemical typing was typical for _M. bovis_ (7).

All smears were positive for acid-fast bacilli except those made from untreated milk and from DTA which developed turbid growth in 1 to 4 days.

The reproducibility of tests was measured in terms of agreement of results in duplicate tests. Of 120 comparisons, 111 (94.9%) were in agreement (similar appearance times), six (5.1%) were in disagreement (one test with growth, one without), and three were eliminated because of contamination.

The data collected in this study revealed the individual influence of four test factors used in evaluating the tuberculocidal properties of two disinfectants. Exposure temperature, exposure time, and disinfectant concentration were important but the effect of _M. bovis_ strain on sensitivity to disinfectants was apparently unimportant. Decontamination was more effective at 23 than at 4 C, thus eliminating the expense of mechanically cooling the unmarketable milk before treatment with disinfectants.

Because the effectiveness of disinfectants increased with greater exposure time, further increases in tuberculocidal activity might be expected with exposures beyond 6 h. However, in actual practice additional exposure time could be impractical. The 1- and 6-h exposure times were selected because the milking schedules of many dairy farms allow 6 h for decontamination plus 2 h for preparing equipment for the next milking.

In preliminary tests, the incubation period for test cultures was 11 days; a period which was considered adequate for detecting growth in untreated subcultures of _M. bovis_. However, in subsequent tests it became apparent that growth of many treated _M. bovis_ cultures was only delayed and a false kill would have been recorded in tests using an 11-day incubation period. An adequate incubation period was determined to be 36 days, after conducting incubation periods up to 63 days. It should be noted that in three instances the first appearance time of _M. bovis_ in DTA was 36 days; however, all three were in the 4 C temperature category which is not recommended for actual use. The intervals between O.D. readings were unequal and could cause a misconception of the actual time involved. For example, if the actual appearance time of a culture was 16 days, it would have been recorded as 22 days, the next reading date.

It has been reported that small amounts of milk, serum, feces, or similar matter in the test environment can effect reductions of up to 90% of the bacteriocidal activity of phenol (6). In this study all of the milk was obtained under ideal conditions from one cow. It is not known whether or not milk from other sources would have altered the results.

Foaming and gelation were observed when Environ was mixed with milk, butphenol caused neither foaming nor gelation. Although no erratic results were specially noted when gelation was conspicuous, the consistency of the clots suggested that contact between disinfectant and _M. bovis_ within the clots was probably restricted.

The data presented here indicate that Environ or phenol could be used in the chemical decontamination of milk from tuberculous cows.

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**REFERENCES**

Tapeworms, Meat and Man: A Brief Review and Update of Cysticercosis Caused by *Taenia Saginata* and *Taenia Solium*

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**ABSTRACT**

Three species of tapeworms may be transmitted to man by ingestion of animal flesh: *Taenia saginata*, *Taenia solium*, and *Diphyllobothrium latum*. The first two are the subject of this brief review which concentrates on recent studies in the field and emphasizes concepts of importance in detection, control, and prevention of cysticercosis. *T. saginata* cysticercosis in beef (beef measles) continues to be a concern in developed countries such as the United States, as well as in developing areas such as East Africa where the infection is widespread. The high standards of meat inspection in the United States have not succeeded in eliminating beef cysticercosis which is seen primarily in feedlot cattle originating in the southwestern U.S. However, it should not be viewed as a strictly regional problem, due to the widespread movement of animals and meat within the United States. Beef cysticercosis is costly due to the special treatment required of infected carcasses; serious effects on human health are rare. In contrast, *T. solium* cysticercosis in swine (pork measles) is rarely reported in areas such as the U.S., Canada, and most European countries, but is still a definite problem in Mexico, some other Latin American nations, and parts of Africa and Asia. As well as being a financial burden, it is a serious public health threat in those countries where it is prevalent. *Taenia solium* is a serious public health threat in those countries where it is prevalent.

Three species of tapeworms may be transmitted to man by ingestion of animal flesh: *Taenia saginata*, *Taenia solium* and *Diphyllobothrium latum*. The first two are the subject of this brief review, which concentrates on recent studies in the field and emphasizes concepts of importance in detection, control and prevention of cysticercosis.

*T. saginata* cysticercosis in beef (beef measles) continues to be a problem in developed countries such as the United States, as well as in developing areas such as East Africa where the infection is widespread. The high standards of meat inspection in the United States have not succeeded in eliminating this infection which is seen primarily in feedlot cattle in the southwestern U.S. It should not be viewed as a strictly regional problem, however, due to the movement of animals and meat within the country. In one outbreak of cysticercosis in a Texas feedlot (44), 7,568 potentially infected cattle were slaughtered at 20 different establishments in eight different states and the meat was then marketed across the country from California to Rhode Island. The problem is primarily an economic one resulting from costly special treatment of infected carcasses. Serious effects on human health are rare.

*T. solium* cysticercosis in swine (pork measles) is rarely reported in areas such as the U.S., Canada, and most European countries, but is still a definite problem in Mexico, some other Latin American nations, and parts of Africa and Asia. As well as being a financial burden, it is a serious public health threat in those countries where it is prevalent. Much less information is available on *T. solium* cysticercosis than *T. saginata* cysticercosis, perhaps because research funding is less readily available for problems not directly affecting developed countries.

**HOSTS, SYMPTOMS, AND PREVALENCE OF INFECTION**

The life cycles of the two tapeworms are relatively simple and quite similar. The adult tapeworms are obligate parasites of the human intestine, and are not found in the adult form in any other species of animal in nature. The adult worms produce gravid segments which leave the human host via the intestinal tract. If gravid segments or eggs are ingested by a suitable intermediate host, the eggs hatch and eventually become larval tapeworms (cysticerci) in the muscles of that animal. The cycle is completed when a human ingests these cysts in the flesh of an intermediate host. The cycle will not be completed if the cysticerci have been rendered nonviable by freezing or adequate cooking of the meat before it is eaten.

The larval cysticerci are considerably less host-specific than the adult worms, and may be found in a variety of intermediate hosts. Domestic cattle are the usual intermediate hosts for the cysticerci of *T. saginata*, but other animals such as buffalo, African wild animals (27) and reindeer in the USSR (2) may be infected. The pig is the usual intermediate host for the larval stage of *T. solium*, but cysticerci have been reported in man and...
other primates, wild carnivores and domestic dogs, wild hogs, deer, sheep and cats (18). Only in massive infections is illness noted in the intermediate host.

Despite the prodigious size of the adult tapeworm (T. saginata) commonly grows to lengths greater than 5 meters, while T. solium is generally smaller), the effect on human health is usually slight, and symptoms are often vague or absent. For T. saginata the most frequent symptom is discharge of a proglottid which may result in a crawling sensation in the perianal region; less commonly symptoms such as abdominal pain, nausea and weakness may be noted (29). Rarely, serious complications result from lodgement of a segment in an aberrant site such as the appendix. While the adult T. solium tapeworm is generally innocuous, man can also become infected with the larval cysticerci, thus serving as both intermediate and definitive host for T. solium. Human infection with cysticerci of T. solium can be fatal as the brain is a frequent site of localization. It is for this reason that T. solium is considered a serious threat to human health even though the adult tapeworm itself seems relatively harmless.

Information on the prevalence of T. saginata infection in man is sparse, but more complete data for presence of the larval cysticerci in cattle at slaughter indicate that the prevalence of infection has increased since World War II in many countries (1, 29, 46). Pawlowski and Schultz (29) reported that the prevalence in Europe in general rose after World War II, and Silverman (47) noted the sharp rise in Britain after the war. The reasons for this apparent increase are not clear, but several hypotheses have been advanced, such as the upheaval and displacement of the human population due to the war, the increased consumption of beef, especially raw beef, and the overtaxing of sewage treatment facilities in many countries. In the United States, U.S. Department of Agriculture statistics (55) show that in federally inspected slaughter houses for the fiscal year 1976, that of approximately 37,000,000 beef inspected, 135 were condemned for cysticercosis, and 9,628 less severely affected carcasses were passed after freezing. It is noteworthy that in the past, over 80% of parasitized cattle have originated from California, Arizona, New Mexico and Texas, the four states bordering Mexico (43). The prevalence of infection in California is 20 times that for the rest of the nation (43). Whether this higher prevalence of infection is due to infected workers from Mexico, importation of Mexican cattle as feeders, the climate, use of irrigation water, and /or the numbers and management of the cattle feedlots in the region is not entirely known. T. saginata cysticercosis is also prevalent in less highly developed regions. Six Latin American countries reported a combined prevalence of 0.27% from 1968-1973 (41) while in some African countries the prevalence may be about 10% (29). Pawlowski and Schultz (29) make the interesting observation that T. saginata is a problem in poor countries because they are poor, i.e. with comparatively lower standards of hygiene, and in rich countries because they are rich, i.e. with increased beef consumption and overtaxed sewage treatment facilities.

T. solium cysticercosis, on the other hand, remains a problem of less highly developed countries where sanitary conditions and systems of pig raising permit direct human to pig transmission. In the United States only four of approximately 70,000,000 hogs slaughtered in federally inspected slaughter-houses were reported to be infected with cysticercosis during the fiscal year 1976 (55). In Mexico, however, 8,820 of 814,000 pigs (1.1%) slaughtered in Mexico City during 1970 were infected (42). Schenone (40) reported that of approximately 10,000,000 pigs slaughtered in some abattoirs of Central and South America between 1960 and 1973, 1.9% were infected with cysticercosis. Acha and Aguilar (3) found that cysticercosis was responsible for 68% of 17,100 condemnations of slaughtered pigs in six Central American countries during 1959-1961. Likewise, T. solium cysticercosis remains a serious problem in parts of South Africa, and in India and other Asian countries.

MECHANISMS OF TRANSMISSION

Man acquires the adult tapeworm by ingestion of raw or inadequately cooked meat containing cysticerci. Beef and pork are the usual vehicles for T. saginata and T. solium, respectively, but dog meat may transmit T. solium in countries where it is eaten (59). Adult worms appear to be long-lived; the length of life of T. saginata appears to be limited only by that of the host (29) and T. solium may live in excess of 10 years (52). Both are prolific egg producers. T. saginata may release 6-9 proglottids daily, containing an approximate total of three-quarters of a million eggs (30); T. solium may extrude five segments daily, containing a total of 250,000 or more eggs (61).

Under proper conditions taeniid eggs can survive for long periods and are resistant to most chemical disinfectants. Sufficient moisture seems to be the most important factor to maintain viability, and eggs survive longer at lower than at higher temperatures. For example, eggs of T. saginata have been kept alive for at least 168 days at 4-5 C (9), and some maintain that they can survive for even longer periods. Jepsen and Roth (19) found that T. saginata eggs survived at least 16 days at 18 C in a dish filled with liquid manure, for 71 days in liquid manure in an underground cistern, and up to 159 days on grass. Recent studies (4) have suggested that eggs when shed may be at different stages of their life span, being in a continuing transition from juvenile (non-infective) to mature (infective) to senescent (diminished ability to invade and develop). Higher temperatures speed the process, suggesting differing potentials for transmission at different seasons of the year.

Transmission from infected humans to the intermediate host is usually indirect via contaminated feed or water, but is occasionally direct as in the infection of
newborn calves by contaminated hands of herdsmen in East Africa (53).

In the transmission of *T. saginata* cysticercosis, contamination of cattle feed may occur in a variety of ways such as defecation of field workers in vegetable or hay fields, or in storage areas for grain, hay, silage, and other feeds used in cattle feedlots. Several recent outbreaks in feedlots in the Southwestern U.S. have been traced to feed contaminated in this manner (44, 50). It is important to note, however, that the act of defecation or the presence of feces may not be necessary to transmit the infection. Proglottids and eggs are usually passed in the feces, but the proglottids of *T. saginata* may be motile and may pass through the anus and expel eggs on the perianal skin (36). Schultz (44) hypothesized that infection of feedlot cattle could have occurred by proglottids from an infected worker dropping unobserved to the ground, thus contaminating cattle feed.

Water can also be a vehicle for transmission of cysticercosis. Infected workers may defecate in irrigation water for crops used as animal feed, or animal drinking water may itself become contaminated. In tests with 10 persons carrying *T. saginata*, Ockert (28) recovered up to 68 oncospheres from fingernail dirt, up to 700 from water for hand washing, and over 50,000 per liter from water used to soak underwear. Effluent from sewage plants is an important mechanism of transmission of cysticercosis. Silverman (48) showed that tapeworm eggs can survive most urban and rural sewage treatment processes and can then pass on in the final effluent or in air-dried sludge; even under ideal circumstances sedimentation and rapid sand filtration will not remove all eggs. Many sewage plants now are old and overworked; increasing numbers of users and increased water usage per user add to the problem, as does the increased use of detergents and other agents which interfere with sedimentation, putrefaction, and oxidation (48). Recent outbreaks of cysticercosis in the U.S. (44) and Australia (34) have been traced to sewage effluent. It must be emphasized that use of sewage effluent in agriculture is not without considerable danger.

Birds have been implicated in transmission of the disease, apparently by ingesting the eggs in sewage or sewage effluent, and by disseminating them in their feces. Gotzsche (16) was able to infect calves with seagull droppings, and it has been suggested that other species of birds may also be involved in transmission of *T. saginata*.

Intrauterine infection of calves with *T. saginata* has been reported (26, 29, 49) but is of unknown importance in the epidemiology of the disease.

Transmission of *T. solium* between man and pigs appears to be less subtle. *T. solium* proglottids do not seem to be motile, and are frequently shed as connected segments presenting less opportunity for dispersion and a greater likelihood of massive infection in a single intermediate host such as a scavenging pig which consumes human feces.

**DEVELOPMENT AND SURVIVAL OF THE CYSTICERCUS AND HOST IMMUNITY**

Under proper circumstances, ingestion of an infective egg by the proper intermediate host will give rise to a larval cysticercus in the muscle of the host. McIntosh and Miller investigated development of *T. saginata* cysticerci in muscles of cattle. At 11 days post infection, the cysticerci and surrounding connective tissue were 3 x 2 mm in diameter and visible to the naked egg. At 18 days they were 4 x 2 mm. They are believed to be infective at 10-12 weeks and are fully developed at about 16 weeks when they may be about the size of a large pea (5 x 8 mm). Cysticerci of *T. solium* may attain approximately the same size. There is much variation in the size of cysts, and size alone cannot be used as a criterion of age. The date of infection can be estimated for young cysts, being accurate only until approximately 10 weeks post-infection for *T. saginata* (25).

The longevity of cysts is variable even within the same animal, and may be partially dependent on the tissue invaded (51, 57) and host age at time of first infection (I). It is not unusual to find both living and dead cysts in the same animal (7, 20, 31). *T. saginata* cysticerci have been shown (57) to live for as long as 3 years after experimental infection. Detailed data are not available for cysticerci of *T. solium*, but degenerated cysts are seen less commonly in pigs than in cattle (59), perhaps because pigs are usually slaughtered at an earlier age than cattle.

In contrast to the adult tapeworm, the larval cysticercus produces an active immune response, perhaps because of its more intimate association with host tissues (62). This aspect of the biology of *T. saginata* and *T. solium* is currently receiving a great deal of attention, and much of the work in the field is concerned with the study of immunity to infection, to development of a vaccine, and to development of procedures which will permit accurate detection of infection in the living animal.

How and when cysticerci produce an immune response in the intermediate host is at best incompletely understood. A complicating factor is the ability of cysticerci to survive in the tissues of immune animals; this has become a central issue in immunoparasitology (15). Calves experimentally infected a few days to several months after birth with a single dose of *T. saginata* eggs will harbor cysticerci from that infection, and are susceptible to reinfection (10-13). However, if calves receive multiple doses of eggs from birth, or if they are infected at 3-4 months of age, they develop a strong resistance to reinfection at a later date (10-13, 51) even though cysticerci from the original exposures may still be present.

**HUMAN INFECTION**

Since infection in man with the adult tapeworm of *T. saginata* or *T. solium* is frequently not accompanied by
clinical signs, diagnosis frequently depends on laboratory examinations. Although eggs can be detected by fecal examination, this method is considered unreliable. The favored diagnostic method is the adhesive cellulose tape or paddle and swab procedure in which a sticky surface is pressed to the perianal skin causing the eggs to adhere to it. The procedure should be repeated several times over several days and will not detect all infections. It is not possible to visually distinguish eggs of *T. saginata* from those of *T. solium*. Distinction between the two species based on segments of the tapeworm is difficult and controversial (32).

The treatment of choice for the adult tapeworm is niclosamide (Yomesan), a safe and effective drug (29). As previously mentioned, man can be infected with the larval cysticercus as well as the adult tapeworm of *T. solium* and can thus act as both definitive and intermediate host. Human infection with the cysticerci of *T. solium* occurs either by ingestion of contaminated food or drink or by auto-infection, in which it is presumed that mature proglottids from an established adult *T. solium* are carried by reverse peristalsis from the small intestine to the stomach where they are stimulated to hatch and subsequently invade the extra-intestinal tissues to become cysticerci. The relative importance of auto-infection is not known, but it would seem reasonable to suppose that pork eaters have a greater chance of acquiring the tapeworm and hence would also have a greater chance of developing *T. solium* cysticercosis if auto-infection were of major importance. Acha and Aguilar (3) found no evidence of an increased prevalence of clinical cysticercosis in individuals parasitized with the adult *T. solium* as opposed to those free of it, and Heinz and Macnab (17) found chances of a non-pork eater becoming infected with cysticercosis to be as great as those of a pork eater. Human illness due to *T. solium* cysticercosis is a significant problem in many countries. For example, in 1972 Biagi (5) reported that human cysticercosis was found in 3-4% of autopsies in Mexico City, and for fully half of these it was the principal disease and probable cause of death. Many clinical pictures are seen, depending on the sites of localization of the cysticerci, and diagnosis can be quite difficult. Serological tests may be used as an aid to diagnosis; the indirect hemagglutination test is employed at the Center for Disease Control (39).

**DETECTION, CONTROL AND PREVENTION**

The life cycles of both *T. saginata* and *T. solium* are relatively simple; man transmits the infection to an animal which in turn transmits the infection to man. It is logical that the chain of infection may be broken in two places: the transmission from man to animal or the transmission from animal to man.

**Man to animal**

One method of halting the transmission from man to animal is to diagnose and eliminate human infections. Programs of mass diagnosis and treatment aimed at eliminating the adult tapeworm from infected persons have reportedly had some success in Bulgaria, Poland and parts of the U.S.S.R. (29). Another measure that has been suggested (45) is the routine checking of feedlot workers for taeniasis before employment. Another method is proper protection of animal feed and water from human feces or from sewage treatment plant effluent.

Because a strong immunity is provoked by the cysticercus, animal vaccination has been investigated as a means of control; successful vaccination of the intermediate host would break the man-to-animal cycle of infection. Most recent work has been done with cattle and *T. saginata*. Immunization in cattle has been achieved by giving irradiated eggs by mouth (54), by the intramuscular injection with onchospheres (14,63), by induction of heterologous immunity with *Taenia hydatigena* (63), by inoculation with a homogenate of *T. saginata* strobila (14) and by a “parasite free” vaccine of antigens produced during in vitro cultivation of cysticerci (33,35). Many of these vaccines produce an excellent immunity to later challenge with *T. saginata* eggs. Young calves, however, do not respond to vaccination, and would be susceptible to infection for the first months of life under a program of control by vaccination. Intramammary vaccination to elicit maternal immunity which would be passed to the calf via colostrum has been accomplished (24). It has been recently reported that a single vaccination of heifers with *T. saginata* culture antigens “parasite free” vaccine during the last month of pregnancy resulted in the transfer of a considerable degree of Colostral immunity to young calves. These calves, when subsequently vaccinated at 8-10 weeks of age, achieved a high degree of immunity to infection (35).

**Animal to man (T. saginata)**

To prevent the animal-to-man transmission of *T. saginata*, it is necessary to first detect infection in cattle either before or after slaughter, and then to prevent human consumption of infective meat.

Visual observation of cysticerci in slaughtered animals by meat inspectors is the most practical and widely used method of detection presently available. Current meat inspection procedure for federally inspected slaughterhouses in the U.S. is multiple incisions in the cardiac muscle and muscles of mastication together with visual examination of the muscle surfaces exposed by splitting the carcass. If one or more cysts are found a more extensive search is undertaken consisting of incisions into each round and into each forelimb 2 or 3 inches above the elbow. Selection of inspection sites is based on the likelihood of finding cysticerci, but there is a diversity of opinion regarding predilection sites for the organism. In addition, it is important for the meat industry that incisions be kept at a minimum and that mutilation of valuable cuts of meats be avoided. It is generally accepted that the muscles of mastication and the heart...
are prime targets for *T. saginata* cysticercosis. In a recent study of feedlot cattle from the Southwest U.S., Juranek et al. (20) found that the heart and muscles of mastication were the most profitable sites in terms of numbers of cysticerci per pound of lean meat, and that the diaphragm was not as helpful. Investigators in South Africa and Rhodesia (37,59) have emphasized the importance of examining the shoulder muscles above the elbow. Robinson (37) reported that in 1,810 cases of *T. saginata* seen in slaughter cattle in Rhodesia 41.8% showed cysts only in the shoulder muscles. There is probably no universally appropriate predilection site. Rather, there may be differences due to geographic area, breed of cattle, age and activity of muscle groups; cysts have been hypothesized to settle in deeper muscles with a higher level of metabolic activity and greater blood supply (22).

Postmortem inspection for detection of *T. saginata* cysticercosis has serious drawbacks. For example, cattle frequently harbor only a few cysticerci, and current inspection procedures miss many light infections. Dewhirst et al. (8) reported that an inspector using the regular incision sites (masticatory muscles and heart) missed 22 of 80 infected carcasses, even though he was expecting to encounter cysticercosis. In addition, the infection is often detected months after it has occurred, making determination of the source difficult. If the source of infection were continuously operating, many animals could be infected in the long lag period between the start of the contamination and the first detection of infection at slaughter.

Use of antemortem techniques for detection could theoretically circumvent some of the defects of postmortem detection and current research efforts are being made in this direction. Serological methods such as complement fixation, latex, indirect hemagglutination, immunofluorescence, and gel diffusion techniques (1) have been tried for the antemortem diagnosis of cysticercosis but at present no good serologic method is available (15,21,29). There is no problem in detecting specific antibodies in heavily infected experimental animals, but the specificity of serologic tests in naturally infected animals is low, and the level of cross reactivity with other cestode species is high (15,21). The enzyme-linked immunosorbent assay (ELISA) technique, a highly promising test for serodiagnosis of parasitic infections, may find application for the detection of *T. saginata* cysticercosis (60). Intradermal tests have been tried, and Dewhirst et al. (6), using saline extracts of lyophilized and defatted portions of adult *T. saginata*, found an accuracy of 75% in naturally infected animals. This method, however, necessitates extra handling of the animals before slaughter to inject the reagent and would require a source of supply of adult *T. saginata*.

Once the cysticerci are detected in an animal, the carcass is condemned if the infection is severe, or in light infections the meat may be subjected to various procedures to destroy the cysticerci. Cysticerci in meat can be killed by a variety of methods and the meat thus rendered safe for human consumption. Freezing at -10 C for 10 days will kill cysticerci in beef (23) and this is the most common current procedure in the United States. Shorter freezing periods would reduce expense, but current studies are lacking (29). Heating beef to temperatures above 56 C will inactivate *T. saginata* cysticerci, as will salting under appropriate conditions, and gamma radiation has also been proposed (29). U.S. Department of Agriculture regulations (56) permit freezing or cooking of lightly infected animals; cattle with one or more live or dead cysts must be frozen at 15 F (-10 C) or less for 10 days, or heated to at least 140 F (60 C). Until 1970, U.S. federal regulations permitted passage of a beef carcass without freezing or cooking if only one dead cyst was found under the assumption that (a) dead cysts occur singly in carcasses and (b) all cysts have the same life expectancy so that if there were more cysts they would also be dead. Minute dissection of 20 heavily infected animals (more than two cysts found on preliminary inspection) and 19 lightly infected animals (one or two cysts) by Juranek et al. (20) has shown these assumptions to be incorrect. Of 11 carcasses where only one cyst was found on initial (routine) inspection, five revealed more cysts on dissection; living and dead cysts were found together in some carcasses. It is obvious that if all beef were adequately cooked before eating that *T. saginata* would be effectively controlled or eliminated, but many people continue to prefer rare or raw beef, and human dietary habits are difficult to change.

### Animal to Man (*T. solium*)

Postmortem detection of cysticercosis in swine is easier than in cattle because lightly infected carcasses are uncommon, and detection is therefore easier. Little work has been done with antemortem serodiagnosis although there are some indications that the ELISA test is not as promising in *T. solium* cysticercosis as in *T. saginata* cysticercosis (60). U.S.D.A. regulations permit pig carcasses with light infections of *T. solium* cysticerci to be passed for cooking (56). On the basis of his studies in Rhodesia, Robinson (38) recently recommended that statutory freezing times for *T. solium*-infected pork carcasses should be reduced to 3 days. Verster (58) has recently stated that infected pork carcasses can be rendered fit for human consumption by exposure to gamma radiation at doses between 20 and 60 Krad. In general, control of *T. solium* infection in both animals and man is more easily effected by postmortem inspection and simple hygienic measures than is *T. saginata* infection.

### RESEARCH TRENDS

At meetings in Germany in 1974 and in Kenya in 1976, experts from around the world discussed current problems in cysticercosis and identified certain areas where research was to be encouraged. The following are...
some of the research needs identified (I):

1. Purification and isolation of species-specific antigens and their evaluation with techniques such as the enzyme-linked immunosorbent assay (ELISA), the soluble antigen fluorescent antibody test, immunolectrophoresis, and counter-current electrophoresis.

2. In vitro culture of various developmental stages for the production of diagnostic and immunizing agents.

3. In vitro or laboratory animal model techniques to assess the infectivity of eggs of *T. saginata* and *T. solium*.

4. Factors that modify survival of eggs.

5. Chemical agents that have ovicidal effect during processing of sewage.

6. The contribution of prenatal and neonatal infection to survival of cisticerci in cattle.

7. The prevalence of antenatal infection in endemic areas.

8. Factors involved in the survival of cisticerci.

9. Active immunization by means of homologous and heterologous species of cestode.


This list provides a good indication of fields in which research is now being carried out and probable areas of future concentration.

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Significance of Mycotoxins to Food Safety and Human Health

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ABSTRACT

Mycotoxins are toxic substances produced by molds, which cause disease in animals or man. Acute diseases caused by mycotoxins are called mycotoxicoses. History has recorded several human disease outbreaks and numerous animal poisonings thought to be mycotoxicoses. The outbreak of Turkey X disease in England in 1960 culminated in the discovery of aflatoxins and the realization that low levels of mold metabolites in foods and feed could cause disease in man and animals. This gave great impetus to the study of mycotoxins. Mycotoxin-producing molds are quite ubiquitous and frequently contaminate food and agricultural commodities. Fortunately, the mere presence of a toxic mold in food does not automatically mean the presence of mycotoxins. Mycotoxins currently receiving the most attention as potential hazards to human and animal health include aflatoxins, ochratoxin A, sterigmatocystin, patulin, penicilllic acid, citrinin, zearalenone and the toxic trichothecenes. These compounds all cause some degree of acute toxicity when given in high amounts. In addition, aflatoxins, sterigmatocystin, patulin and penicilllic acid are potential carcinogens.

The significance of mycotoxins as causes of human diseases is difficult to determine because there is no direct evidence of such involvement in terms of controlled experiments with man. Human cases of ergotism and alimentary toxic aleukia are known to be of fungal origin. Recent reports have linked aflatoxins to acute poisonings of humans in Africa, southeast Asia and India. Epidemiological studies have correlated aflatoxin contamination of foodstuffs with high incidences of liver cancer and other liver disease in certain regions of the world. It has been suggested that ochratoxin A may be involved in a fatal kidney disease of humans known as Balkan Endemic Nephropathy. Ochratoxin A has been found in foodstuffs from the endemic areas of this disease.

Mycotoxins may enter the food supply by direct contamination, resulting from mold growth on the food, or by indirect contamination through the use of contaminated ingredients in processed foods. Indirect exposure to mycotoxins can also result from consumption of animal products, such as milk, which contain mycotoxin residues, caused by feeding moldy feed to the food-producing animal. Commodities susceptible to direct contamination with mycotoxins include nuts, oilseeds, grains and to a limited extent, certain fruits. Residues of aflatoxin have been found in animal products such as fluid milk, nonfat dry milk, cottage cheese and imported cheeses. In feeding experiments with aflatoxins, the toxins were found in livers, kidneys and certain tissues of pigs and broiler chickens, and in eggs from laying hens fed aflatoxin. Residues of ochratoxin A have been found in livers, kidneys, muscles and adipose tissues of bacon pigs and poultry. Refrigerated foods, such as cheeses, cured meats and certain flour-based products, subject to mold growth during storage, have been shown to be contaminated with a variety of potential mycotoxin-producing molds. Experimental evidence indicates that certain mycotoxins could be produced on refrigerated foods under certain conditions. Aflatoxin production is favored by temperatures of 20 to 25 C, but has been reported to occur as low as 7 to 12 C. Toxins produced by Penicillium species can be produced at temperatures as low as 5 C; however, patulin and penicilllic acid do not appear to be produced to any extent on substrates such as cheeses and cured meats. Aflatoxins and ochratoxins appear to be relatively stable in most foods, whereas patulin and penicillic acid are not stable in proteinaceous foods such as cheeses and meats. Stability data on other mycotoxins are lacking for most foods. In general, mycotoxins are most stable in grains, nuts and oilseeds. The current tolerance level for aflatoxins in foods is 20 ppb, which will probably be lowered to 15 ppb in the near future. Recently, an action level of 0.5 ppb for aflatoxin in milk and milk products was announced which is essentially a tolerance level for these products.

Mycotoxin is a general term used to describe compounds or metabolites, which are toxic or have other biological effects in living organisms (primarily animals and/or man), and which are produced by molds. The term is derived from the Greek words "mykes" meaning fungus and "toxicum" meaning poison or toxin (60). Thus, the term literally means fungus poison or fungus toxin. A number of the compounds which are today classed as mycotoxins were actually first studied as potential antibiotics in the 1930's and 1940's, only to be discarded as being too toxic to higher life forms to be of value in treating disease. At that time the potential health problems that these compounds might pose as contaminants of the food supply was not recognized.

The acute diseases caused by mycotoxins are referred to as mycotoxicoses. History has recorded a number of outbreaks of human mycotoxicoses. In addition, the scientific literature contains many more references to disease outbreaks among domestic animals that were
either proven or suspected of being caused by mycotoxins. Outbreaks of ergotism have been recorded in Europe as far back as the Middle Ages (60). This disease, also known as “St. Anthony’s Fire,” killed thousands of people in France in 943 A.D. It wasn’t until the 19th century that the cause of this disease was recognized as a group of alkaloid compounds produced by the fungus Claviceps purpurea, commonly known as ergot, which parasitizes rye and other grasses. A human disease known as Alimentary Toxic Aleukia (ATA) occurred in the Orenburg Province of Russia during World War II (77). The disease, caused by consumption of overwintered moldy grain, was manifested by severe dermal necroses, hemorrhaging, leucopenia (abnormal decrease in leukocytes) and bone marrow degeneration. Mortality rates were as high as 60% in some instances, with up to 10% of the population being affected. Several molds were subsequently shown to be involved in the etiology of the disease, including Fusarium poae, Fusarium sporotrichoides and several Cladosporium species. About this same time, a disease of horses known as Stachybotryotoxicosis also occurred in Russia (54). This disease was caused by the feeding of moldy hay containing the mold Stachybotrys alternans (atra). Also, about this time in history, the Japanese recorded a condition known as “yellowed rice” which caused serious liver damage when ingested by animals (140). Subsequently, several compounds known as yellow rice toxins were isolated. It was found that a number of Penicillium species, notably Penicillium citreo-viride, Penicillium citrinum, Penicillium islandicum and Penicillium rugulosum were involved. Much of the Japanese’s detailed work was published in the early 1950’s, yet the field of mycotoxins received very little attention until 1960. In that year a severe toxic outbreak occurred in England, which became known as “Turkey X Disease” because of the involvement of large numbers of turkey pouls (10). In addition, ducklings and other young farm animals were also affected (4). The cause of the disease was traced to a feed component, peanut meal, which was heavily infested with the common storage mold Aspergillus flavus. Analysis of the feed led to discovery of a series of fluorescent compounds which were named aflatoxins, for A. flavus toxins. At about the same time, an outbreak of trout hepatoma was observed in the U.S. (180). This was later related to aflatoxin-contaminated cottonseed meal used in the diet of the trout (65).

Further study of the effects of aflatoxins in animals revealed that these compounds were toxic to a wide range of animals and that the effects observed varied, depending among other things on the dosage given (1,2,178,179). In large doses aflatoxins were found to be acutely toxic, causing gross liver damage with intestinal and peritoneal hemorrhaging, resulting in death of the animal. Sub-lethal doses resulted in a number of moderate to severe histopathological changes in the liver, such as necrosis, hemorrhage, chronic fibrosis, bile duct hyperplasia and fatty degeneration. Chronic exposure of rats to low levels of aflatoxins revealed that the compounds were carcinogenic and were capable of inducing liver tumors (95,178,179). These studies showed aflatoxin B₁ to be the most potent carcinogen known, when it was found that microgram quantities were capable of producing tumors in a high percentage of test animals. This led to the first realization that chronic exposure to low levels of mold metabolites could cause disease in man and animals.

The early work with aflatoxin gave great impetus to the study of mold metabolites as possible disease agents and potentially harmful contaminants in food and feed supplies. To date, studies have shown that a number of additional mycotoxins also exhibit the properties of acute, sub-acute and chronic effects in animals with some also being carcinogenic. In addition, some mycotoxins are also now known to be mutagenic, capable of causing mutations in susceptible organisms (which may also suggest carcinogenicity) and teratogenic, capable of causing deformities in developing embryos.

**MYCOTOXIN-PRODUCING MOLDS IN FOODS**

Many of the molds capable of producing mycotoxins are also frequent contaminants of food and agricultural commodities. Molds which are of importance in foods because of potential mycotoxin production include members of the genera Aspergillus, Penicillium, Fusarium, Alternaria, Trichothecium, Cladosporium, Bysscohlamys and Sclerotinia. These organisms are capable of growth on a variety of substrates and under a diversity of conditions of moisture, pH and temperature. Thus, most foods are susceptible to fungal invasion during some stage of production, processing, transport or storage. If mold growth occurs, there is always the concomitant possibility of mycotoxin production. However, the presence of toxigenic molds in a food product does not automatically mean the presence of mycotoxins, especially if growth has not occurred, but rather that a potential for mycotoxin contamination exists. On the other hand, the absence of toxigenic molds does not guarantee that the commodity is free of mycotoxins, since the toxins may persist long after the molds have disappeared.

Numerous studies have reported the incidence and types of toxigenic molds on various food and agricultural commodities (Table 1). These studies indicate the ubiquitous distribution of potential mycotoxin-producing molds. The types of molds present in a commodity are affected by such factors as the substrate, moisture and storage conditions. The mycoflora of cereal grains, for example, can be divided into three groups: (a) field fungi, which invade the grain in the field before harvest, and include species of Alternaria, Fusarium, Helminthosporium and Cladosporium; (b) storage fungi, which predominate in grains during storage after harvest and consist primarily of species of Aspergillus and Penicillium; (c) advanced decay fungi, such as Fusarium and Chaetomium, which grow after considerable damage
### TABLE 1. Summary of some selected reports of isolations of potentially toxic molds from various food or agricultural commodities.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Potentially toxic genera/species found</th>
<th>Potential mycotoxins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour, bread, cornmeal, popcorn</td>
<td>Aspergillus flavus, ochraceus, versicolor</td>
<td>Penicillium citrinum, citreo-viride, cyclopium, martensii, patulum, puberulum</td>
<td>16, 22, 25, 63</td>
</tr>
<tr>
<td>Peanut, in-shell pecans</td>
<td>Cladosporium, Fusarium Aspergillus flavus, parasticus ochraceus versicolor</td>
<td>Penicillium cyclopium, expansum citrinum</td>
<td>5, 49, 101, 141</td>
</tr>
<tr>
<td>Apples and apple products</td>
<td>Fusarium, Rhizopus, Chaetomium Penicillium expansum</td>
<td>Patulin</td>
<td>12, 155</td>
</tr>
<tr>
<td>Meat pies, cooked meats, cocoa powder, hops, cheese</td>
<td>Aspergillus flavus Cladosporium</td>
<td>Penicillium viridicatum roqueforti patulum commune</td>
<td>17, 18, 19</td>
</tr>
<tr>
<td>Aged salami and sausage, country cured ham, moldy meats</td>
<td>Aspergillus flavus ochraceus versicolor</td>
<td>Penicillium viridicatum cyclopium</td>
<td>20, 21, 52, 96, 162, 163, 164</td>
</tr>
<tr>
<td>Black and red pepper, macaroni</td>
<td>Aspergillus flavus ochraceus versicolor</td>
<td>Penicillium species</td>
<td>29, 30, 32</td>
</tr>
<tr>
<td>Dry beans, soybeans</td>
<td>Aspergillus flavus ochraceus versicolor Alternaria Cladosporium</td>
<td>Penicillium cyclopium viridicatum citrinum expansum islandicum urticae</td>
<td>110, 111</td>
</tr>
<tr>
<td>Refrigerated and frozen pastries</td>
<td>Aspergillus flavus versicolor</td>
<td>Penicillium cyclopium citrinum martensii olivina-viride patulum puberulum roqueforti urticae viridicatum</td>
<td>92, 93</td>
</tr>
<tr>
<td>Moldy supermarket foods</td>
<td>Penicillium cyclopium Aspergillus species</td>
<td>Penicillic acid, T-2, possibly other Penicillium toxins</td>
<td>45</td>
</tr>
<tr>
<td>Moldy supermarket foods</td>
<td>Fusarium oxysporum solani Aspergillus species</td>
<td>Penicillic acid, A. patulin, ochratoxin</td>
<td>166</td>
</tr>
</tbody>
</table>

from other microorganisms has occurred (29,31). Field fungi and advanced decay fungi require moisture levels of 20 to 25% to grow, whereas storage fungi can grow at moisture levels of 13 to 18%. Recent evidence indicates that *A. flavus* may invade grains, particularly corn, in the field when the grain has suffered insect or hail damage. Most mycotoxic fungi associated with grains and grain products as well as most other foods are species of *Aspergillus*, *Penicillium* and *Fusarium* (74).

### MYCOTOXINS

Many toxic compounds have been isolated from mold cultures. However, not all of these have been shown to have a role in human or animal diseases. The literature concerning mycotoxins has been extensively reviewed in detail in numerous volumes and review articles over the past several years (37, 40, 59, 67, 78, 79, 103, 118, 129, 130, 136, 137, 158, 170, 186, 187). These reviews discuss the known mycotoxins in some depth. For purposes of this
discussion, attention will be directed toward those toxins which may be considered to pose the greatest potential hazard to human health as food contaminants. These toxins include aflatoxins, ochratoxin A, sterigmatocystin, patulin, penicillic acid, citrinin, zearalenone and the toxic trichothecenes. Some of the chemical and physical properties of these mycotoxins are summarized in Table 2, and toxicological and biological properties are summarized in Table 3.

**Aflatoxins**
Aflatoxins are produced primarily by some strains of *A. flavus* and most, if not all, strains of *Aspergillus parasiticus* (48). Aflatoxins are a group of closely related heterocyclic compounds of which six are most common (Fig. 1). There are four main aflatoxins, B₁, B₂, G₁, and G₂. Of these, B₁ and G₁ occur most frequently and in largest amounts. Under long-wave ultraviolet light, aflatoxins B₁ and B₂ fluoresce blue and aflatoxins G₁ and G₂ fluoresce green (Table 2). The B and G designations of the toxins refer to the color of fluorescence. The subscripts 1 and 2 refer to the separation pattern of these compounds on thin-layer chromatography (TLC) plates, with B₁ having the highest Rf value followed by B₂ then G₁ and G₂ in most solvent systems. In addition to these four aflatoxins, two additional toxins are of significance; these are aflatoxins M₁ and M₂. The M toxins were first isolated from the milk of lactating animals fed aflatoxin preparations; hence, the M designation (73). The subscripts again refer to separation patterns on TLC plates (Table 2). The M toxins also fluoresce blue when exposed to long-wave U.V. light, but separate at a lower Rf value on TLC plates than the B and G toxins. Chemically, the aflatoxins are difuranocoumarin derivatives structurally related to coumarin (15).

Aflatoxins are potent hepatotoxins and also potent carcinogens. Aflatoxin B₁ is the most toxic of the group (179). Effects of aflatoxins in vivo vary with dose, duration of exposure, animal species, breed and diet or nutritional status of the animal affected. As mentioned earlier, these toxins may be acutely toxic when given in large doses; sub-lethal doses produce a chronic toxicity and low levels of chronic exposure result in carcinogenic responses in a number of animals. The oral 7-day LD₅₀ values for aflatoxins in ducklings are shown in Table 3. LD₅₀ values for other animals range from 0.5 to 10 mg/kg of body weight (179). Many animal species are affected by aflatoxins. In general, young animals of any species are more susceptible to the acute toxic effects of aflatoxins than are older animals of the same species. Susceptibility also varies between species. With poultry, ducklings are most susceptible followed by turkey poult, pheasant chicks, mature chickens and quail in that order. Among mammals, 3- to 12-week-old pigs are most susceptible followed by pregnant sows, fattening pigs, mature cattle and sheep (1,2). Trout and dogs are also susceptible to the effects of aflatoxins.

The clinical signs of acute aflatoxicosis in most species include lack of appetite, weight loss, unthriftiness, neurological abnormalities, jaundice of mucous membranes, convulsions and death (67). Gross liver damage is also evident, livers being pale or discolored with necrosis and fat accumulation. There may also be fluid accumulation in the body cavity and hemorrhaging of the kidneys and intestinal tract. Sub-lethal, chronic exposure to aflatoxins results in jaundice of the carcass and cirrhosis of the liver, with bile duct proliferation and fibrosis. Prolonged exposure to low levels of aflatoxins results in liver tumors in a number of species including trout, ducklings and rats. Trout are most susceptible to the carcinogenic effects of aflatoxins and develop liver tumors when exposed to only a few parts per billion of aflatoxins.

Aflatoxins have been found in a number of products, particularly peanuts and peanut products, cottonseed and corn. Besides these products, other commodities considered most likely to be contaminated with aflatoxins include copra, Brazil nuts, pistachio nuts, almonds, pecans and walnuts (156).

**Sterigmatocystin**
Sterigmatocystin is produced by *Aspergillus versicolor*, *A. flavus*, *A. nidulans*, *A. rugulosus*, *Penicillium luteum* and a *Bipolaris* species (43,46). Structurally, sterigmatocystin resembles the aflatoxins and is basically a xanthone nucleus attached to a bifuran ring (Fig. 2). Several related compounds also exist, but sterigmatocystin appears to be the most important. It is thought to be a precursor in the biosynthesis of aflatoxin. The acute toxicity of sterigmatocystin is low with an oral LD₅₀ in mice of 800 mg/kg (102). The main concern with sterigmatocystin is that it is carcinogenic when given orally to rats, resulting in liver cancer (132,133). Sterigmatocystin is about one-tenth as potent a carcinogen as aflatoxins, but certain cultures of *A. versicolor* are capable of producing large amounts of the compound. Sterigmatocystin has been detected in low levels in green coffee and moldy wheat (131,144).

**Ochratoxins**
Ochratoxins are a group of related compounds that are produced by *Aspergillus ochraceus* and related species, as well as *Penicillium viridicatum* and certain other *Penicillium* species (33,67,117,186). The main toxin in this group is ochratoxin A (Fig. 3). Chemically, ochratoxin A is a dihydroisocoumarin linked to L-β-phenylalanine (67,186). Ochratoxin A is a potent mycotoxin that causes kidney damage in rats, dogs and swine (Table 3). Ochratoxin is thought to be involved in disease of swine in Denmark known as porcine nephropathy, which has been associated with the feeding of moldy barley (86,88,90). The LD₅₀ values for ochratoxin A (Table 3) in chicks, swine and trout range from 2.1 to 4.67 mg/kg (117,186). The main pathological effects of acute ochratoxicosis are necrosis of the renal tubular epithelium of the kidney and peritubular liver cells with accompanying enteritis (67). Ochratoxin has also been reported to be teratogenic to mice, rats and chicken embryos (117). Ochratoxin has been detected in
### TABLE 2. Some chemical and physical properties of several mycotoxins (28,36,186).

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Mol. Wt.</th>
<th>Approx. R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Solvent system&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Solvent ratio</th>
<th>Fluorescent color in U.V. light (562 nm)</th>
<th>Ultra-violet absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maxima (nm)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>312</td>
<td>0.56</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>223;265;362</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>314</td>
<td>0.53</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>25,600;13,400;21,800</td>
</tr>
<tr>
<td>G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>328</td>
<td>0.48</td>
<td>C:M</td>
<td>97:3</td>
<td>Green</td>
<td>265;263</td>
</tr>
<tr>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>330</td>
<td>0.46</td>
<td>C:M</td>
<td>97:3</td>
<td>Green</td>
<td>243;257;264;362</td>
</tr>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>330</td>
<td>0.40</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>265;263</td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>330</td>
<td>0.30</td>
<td>C:M</td>
<td>97:3</td>
<td>Red-brown (Brick red)</td>
<td>226;265;357</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T:E:F</td>
<td>6:3:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>403</td>
<td>0.55</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Greenish-blue</td>
<td>213;332</td>
</tr>
<tr>
<td>Citrinin</td>
<td>250</td>
<td>0.16-48 (streak)</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Bright blue&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36,800;6,400</td>
</tr>
<tr>
<td>Penicillnic acid</td>
<td>154</td>
<td>0.41</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Yellow</td>
<td>222;253;319</td>
</tr>
<tr>
<td>Zearealenone</td>
<td>318</td>
<td>0.78</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Pale blue&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14,540</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>466</td>
<td>0.36</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Bright blue&lt;sup&gt;c&lt;/sup&gt;</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Faint blue&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10,500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gray-pink&lt;sup&gt;e&lt;/sup&gt;</td>
<td>236;274;316</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29,700;13,909;6,020</td>
</tr>
</tbody>
</table>

<sup>a</sup>C = chloroform; E = ethyl acetate; F = formic acid (90%); T = toluene.

<sup>b</sup>In ethanol unless otherwise noted.

<sup>c</sup>After exposure to ammonia fumes.

<sup>d</sup>In short wave U.V. light, zearealenone fluoresces green and is brighter.

<sup>e</sup>After spray with anisaldehyde.

### TABLE 3. Some biological and toxicological properties of several mycotoxins (28, 36, 186, 187).

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Producing organism</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mg/kg)</th>
<th>Animal</th>
<th>Route admin.</th>
<th>Effects in animals</th>
<th>Commodities found contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Aspergillus flavus parasiticus</td>
<td>0.5-10</td>
<td>Several</td>
<td>oral</td>
<td>Birds</td>
<td>Peanuts, Corn, Wheat, Rice, Cottonseed, Copra, Nuts</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Same</td>
<td>0.36</td>
<td>Duckling</td>
<td>oral</td>
<td>Duckling</td>
<td></td>
</tr>
<tr>
<td>G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Same</td>
<td>1.7</td>
<td>Duckling</td>
<td>oral</td>
<td>Turkey poult</td>
<td></td>
</tr>
<tr>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Same</td>
<td>0.78</td>
<td>Duckling</td>
<td>oral</td>
<td>Pheasant chick</td>
<td></td>
</tr>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Same</td>
<td>3.45</td>
<td>Duckling</td>
<td>oral</td>
<td>Mature chickens</td>
<td></td>
</tr>
<tr>
<td>M&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Same (Also excreted by animals after consumption)</td>
<td>0.32</td>
<td>Duckling</td>
<td>oral</td>
<td>Dogs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.23</td>
<td>Duckling</td>
<td>oral</td>
<td>Mice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of Mycotoxins

Fig. 1

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Note: The table provides detailed information on mycotoxins, including their molecular weights, approximating RF values, solvent systems, fluorescent colors, and ultra-violet absorption maxima. The table also highlights the producing organisms and LD<sub>50</sub> values for acute toxicity, as well as the effects on animals and the commodities found contaminated.
<table>
<thead>
<tr>
<th>Sterigmatocystin</th>
<th>Aspergillus versicolor</th>
<th>800</th>
<th>Mouse</th>
<th>oral</th>
<th>Mouse</th>
<th>Carcinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>flavus</em></td>
<td>120</td>
<td>Rat</td>
<td>oral</td>
<td></td>
<td>Rat</td>
<td></td>
</tr>
<tr>
<td>rugulosus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>B</td>
<td>Same</td>
<td>54</td>
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<td>Swine</td>
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<td>Same</td>
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<td>Chick</td>
<td>oral</td>
<td>Dogs</td>
<td>Porine nephropathy</td>
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| Citrinin        | *Penicillium citrinum* | 110 | Mouse | oral | Swine | Acute kidney |}
|                | *citro-viride* | 35  | Mouse | sc,ip |     | damage      |
|                | *claviforme*  | 38  | Mouse | iv    |     |            |
|                | *expansum*    | 67  | Rat   | sc    |     |            |
|                | *notatum*     | 37  | Guinea pig | sc |     |            |
|                | *viridicatum* | 19  | Rabbit | iv  |     |            |
|                | (several others) |           |       |      |     |            |
|                | *Aspergillus candidus* | 110 | Mouse | oral | Swine | Edema       |
|                | *niveus*      | 35  | Mouse | sc,ip |     |           |
|                | *terus*       | 38  | Mouse | iv    |     |           |
|                | *Clavariopsis Blennoria* | 67  | Rat   | sc    |     |           |
|                | *Aspergillus clavatus* | 37  | Guinea pig | sc |     |           |
|                | *giganteus*   | 19  | Rabbit | iv  |     |           |
|                | *terreus*     | 170 | Mouse | oral | Birds | Edema       |
| Patulin         |                 | 25  | Mouse | iv    |     | Brain       |
|                |                 | 25-50 | Rat | iv    |     | Lungs       |
|                |                 | 35  | Chicken | oral |     |           |
|                | *Penicillium claviforme* | 35  | Mouse | iv    |     | Hemorrhage |
|                | *cyclopium*   | 25  | Mouse | iv    |     | lungs      |
|                | *divergens*   | 170 | Chick | oral |     |           |
|                | *equinum*     |     |       |      |     |           |
|                | *expansum*    |     |       |      |     |           |
|                | *griseofulvum* |     |       |      |     |           |
|                | *lapidoxum*   |     |       |      |     |           |

|                | Birds          |     |       |      |     |            |
|                | Chickens       |     |       |      |     |            |
|                | Chicken embryo |     |       |      |     |            |
|                | Quail          |     |       |      |     |            |
|                | Mammals        |     |       |      |     |            |
|                | Cat            |     |       |      |     |            |
|                | Cattle         |     |       |      |     |            |
|                | Mouse          |     |       |      |     |            |
|                | Rabbit         |     |       |      |     |            |
|                | Others         |     |       |      |     |            |
|                | Brine shrimp   |     |       |      |     |            |

Cereal grains: Wheat, Barley, Oats, Corn, Rice

Nephrotoxin: Tubular necrosis of kidney

Swelling of kidney:

Acute kidney damage:

Tubular necrosis of kidney:

Moldy feed: Moldy feed

Rotted apples: Rotted apples

Apple juice: Apple juice

Wheat straw residue: Wheat straw residue
<table>
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<th>Penicillium Acid</th>
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<th>Mouse</th>
<th>oral</th>
<th>Rat</th>
<th>sc</th>
<th>Mouse</th>
<th>oral</th>
<th>Rat</th>
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<td>Guppies</td>
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<td>1-5 mg/kg (ppm) causes physiological responses</td>
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Zearalenone (F-2)

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<td>oral</td>
<td>Rat</td>
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Data not available but 1-5 mg/kg (ppm) of feed causes physiological responses

- Convulsions
- Carcinogen
- Antibiotic
- Liver damage
- Fatty liver
- Cell necrosis
- Kidney damage
- Digitalis-like action on heart
- Dilates blood vessels
- Antidiuretic
- Edema in rabbit skin
- Carcinogenic
- Antibiotic
- Estrogenic effects
- Swelling and edema of vulva
- Prolapset of vagina
- Enlargement of uterus
- Atrophy of ovaries
- Atrophy of testicles
- Enlargement of mammary glands
- Abortion

- Stored corn
- Cereal grains
- Dried beans
- Moldy tobacco
- Corn
- Moldy hay
- Pelleted commercial feeds
<table>
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<th>Trichothecenes</th>
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<td>Calf</td>
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<tr>
<td></td>
<td>trichitum</td>
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</table>

|                | Swine    | Oral     | Digestive disorders | Corn |
|                | Cattle   | Oral     | Emesis              | Commercial |
|                | Chicken  | Oral     | Diarrhea            | cattle feed |
|                | Turkey   | Oral     | Refusal to eat      | Mixed feeds |
|                | Horse    | Oral     | Hemorrhaging        |       |
|                | Rat      | Oral     | Stomach             |       |
|                | Mouse    | Oral     | Heart               |       |
|                | Dog      | Oral     | Intestines          |       |
|                | Cat      | Oral     | Lungs               |       |
|                | Man      | Oral     | Bladder             |       |
|                |          | Oral     | Kidney              |       |
|                |          | Oral     | Edema               |       |
|                |          | Oral     | Oral Lesions        |       |
|                |          | Oral     | Dermatitis          |       |
|                |          | Oral     | Blood Disorders     |       |
|                |          | Oral     | (Leucopenia)        |       |

\(^a\text{Se} = \text{sub-cutaneous, } ip = \text{intraperitoneal, } iv = \text{intravenous.}\)
nephrotoxic response in laboratory animals similar to swine nephropathy (67,170,186). Citrinin may be involved with ochratoxin A in cases of swine nephropathy in Denmark (88). However, the toxicity of citrinin is low compared to ochratoxin (58,88,91), although possible synergistic activity with ochratoxin A cannot be ruled out. Citrinin has been found to occur naturally in feed samples from farms with swine nephropathy in Denmark, moldy grain in Canada and yellowed rice in Japan (67,140,170). In the feed samples and moldy grain, citrinin was found along with ochratoxin A. In feeding trials with rats, citrinin caused renal damage, with pathological examination showing enlarged, turbid, gray-white kidneys and tubular damage to the kidneys (140).

Patulin

Patulin is an unsaturated lactone (Fig. 5) that is similar in structure to certain other carcinogens (39). In early work with patulin, going back to the 1940's, researchers applied different names to patulin, so several synonyms for this compound exist in the early literature. Other names for patulin are expansin, clavacin, clavatin, claviformin, penicidin, myocin c, gigantic acid, tercinin and leucopin (67,153,158). Patulin is toxic to many biological systems (Table 3), but its role in causing animal and human disease is unclear. Patulin is toxic to many bacterial systems, mammalian cell cultures, higher plants and animals including mice, rats, rabbits, cats, chicken embryos, chickens, quail, guppies, brine shrimp, zebra fish and crustacean Cyclops fuscus (153,158). The oral LD$_{50}$ for patulin in mice is 35 mg/kg (13). Patulin has also been shown to be carcinogenic when injected intradermally into mice in sub-lethal doses (47).

Patulin is produced by numerous Penicillium and Aspergillus species and Byssochlamys nivea (Table 3). Penicillium expansum, which commonly occurs in apple commercial corn and barley and in feed grains and mixed feeds of low quality. Ochratoxin has also been found in dried white beans, moldy peanuts, and barley and oats associated with swine nephropathy, and tissues of these swine (67).

Citrinin

Citrinin (Fig. 4) is a yellow cyclic compound with a free carboxylic acid group (67,140). Citrinin is produced by several Penicillium species including P. citrinum, P. viridicatum, P. expansum and P. notatum, as well as Aspergillus species (67,140,186). Like ochratoxin A, citrinin is a kidney toxin (Table 3). Citrinin causes a
rots produces patulin, as does *P. patulum (urticae)*, *P. claviforme* and various other *Penicillium* species (158, 186). *Aspergillus clavatus*, *A. giganteus* and *A. terrus* also produce patulin (39,158). Patulin is of some public health concern because of its potential carcinogenic properties, and because it has been found in commercial apple juice (143,175,177). Patulin has also been implicated in the deaths of cattle in Japan (189). The toxic effects of patulin in mice include paralysis of the motor nerves, convulsions and reflex excitement. Pathological findings in experimental cases of patulin toxicosis in rats, mice, rabbits and chickens have been reported to be lung edema, with hemorrhaging, capillary damage in the liver, spleen and kidneys, edema of the brain, and liver damage (67,158).

**Penicillic acid**

Penicillic acid is also an unsaturated lactone-type compound, and in solution exists in two tautomeric forms: as the γ-hydroxy lactone ring and as the γ-keto acid (Fig. 6). It is questionable whether penicillic acid should be classified as a toxin since the oral toxicity of this compound is low (Table 3). The oral LD₉₀ of penicillic acid in mice is 600 mg/kg (39,67). However, the concern about penicillic acid in foods is based on the fact that the compound bears structural similarity to known carcinogenic lactones and has in fact been shown to be carcinogenic to rats when injected subcutaneously (47). However, the potency of penicillic acid as a carcinogen is much lower than aflatoxins since an injection dose of 1 mg given twice weekly for 64 weeks was required to produce the cancer (47). When given in lethal doses, penicillic acid caused fatty liver degeneration in quail and liver cell necrosis in mice (42). Mixtures of penicillic acid with ochratoxin A have been reported to give a synergistic lethal response in mice (105). Pharmacologically, penicillic acid dilates blood vessels and has antidiuretic effects.

Penicillic acid (Table 3) is produced by strains of *A. ochraceus* and related species and several *Penicillium* species including *P. cyclopium*, *P. martensii*, *P. palitans*, and *P. puberulum*, among others (39,186). Some strains of *A. ochraceus* are capable of producing penicillic acid along with ochratoxin A (6,35). Recently, Olivigni and Bullerman (123,124) reported the simultaneous production of penicillic acid and patulin by an atypical *Penicillium roqueforti*. Penicillic acid has been found in large quantities in stored corn (41,94).

**Zearalenone**

Zearalenone is an estrogenic compound which is also known as F-2 toxin (Fig. 7). It causes vulvovaginitis and estrogenic responses in swine (109). Zearalenone is produced by *Fusarium* species including *F. roseum*, *F. tricinctum*, *F. oxysporum*, *F. culmorum*, and *F. moniliforme* (27,109,116,125). Zearalenone has been found to occur naturally in high moisture corn in late fall and winter, primarily from the growth of *F. roseum* (109). The compound is quite toxic, with 1 to 5 ppm sufficient to cause physiological responses. In addition to swine, rats, turkey poults and chicks have been shown to be susceptible to the toxic effects of zearalenone (109). Some of the pathological effects of zearalenone in swine include swelling and edema of vulva, prolapal of the vagina, enlargement of the uterus and atrophy of the ovaries in young gilts. Young male swine develop mammary gland hyperplasia and testicular atrophy (109). Zearalenone can be transmitted to piglets in sows' milk and cause estrogenism in the young pigs (67). Zearalenone has been found in moldy hay, high moisture corn, corn infected before harvest and pelleted feed rations (67).

**T-2 toxin**

T-2 toxin (Fig. 8) is one of a family of closely related compounds produced by several *Fusarium* species (8). These compounds are derivatives of a ring system referred to as trichotheccene, and are characterized as 12-13 epoxytrichotheccenes. Their toxicity is attributed to the epoxy group at carbons 12 and 13 and the olefinic bond at carbons 9 and 10 (8). There are more than 20 naturally occurring compounds produced by *Fusarium* species which contain similar structures, including diacetoxyscirpenal, neosolaniol, nivalenol, diacetylnivalenol, deoxynivalenol, HT-2 toxin and fusarenon X (7).
The chemical and biological properties of these compounds have been reviewed extensively by Bamberg (7) and Bamberg and Strong (8). T-2 toxins and related compounds have been implicated with a disease known as moldy corn toxicosis of swine, symptoms of which include refusal to eat (refusal factor), lack of weight gain, digestive disorders and diarrhea, ultimately leading to death. Pathological findings include hemorrhagic lesions in the stomach, heart, intestines, lungs, bladder and kidneys (154). T-2 toxin is quite toxic to rats, trout and calves with oral LD₅₀ values of 3.8, 6.1 and 0.6 mg/kg, respectively (67). Fatal doses of T-2 toxin in chickens have resulted in severe edema of the body cavity and hemorrhage of the large intestine, along with neurotoxic effects and oral lesions (183,184,185). These effects are similar to those observed with moldy corn toxicosis. T-2 toxin is also thought to be one of the toxins involved in the human disease alimentary toxic aleukia (ATA) and stachybotryotoxicosis of horses (8). Recently, Yagen and Joffe (188) reported that isolates of F. poae and F. sporotrichoides from overwintered cereals associated with ATA in the U.S.S.R. produced T-2 toxins in culture. T-2 toxin also causes severe dermal responses in rabbits, rats and other animals, including humans, when applied to the skin. However, T-2 toxin is not thought to be carcinogenic (8).

**RELATION TO HUMAN HEALTH**

The relationships of mycotoxins to human health as the etiological agents of disease syndromes in man are difficult to determine because there is no direct evidence of such involvement in terms of controlled experiments with man. Certainly the diseases of ergotism and alimentary toxic aleukia can now be attributed to fungal toxins. Evidence also strongly suggests that acute cardiac beriberi, common throughout Asia, may in fact be linked to the so-called yellowed rice toxins (citrinin, luteoskyrin, rugulosin, cyclochlorotine and citreoviridin) which are produced by numerous *Penicillium* species isolated from yellowed rice (140). Also, the fact that many different animal species are susceptible to both acute and chronic effects of mycotoxins is strong indirect evidence that man too would most likely be adversely affected in a similar fashion. This is particularly true with those toxins which affect animals which are physiologically similar to man such as primates and swine. While the incidence of mycotoxic disease in animals appears to be higher than in man, this is not surprising and does not mean that animals are necessarily more susceptible to mycotoxins than man. It simply means that animals are more likely to be exposed to mycotoxins than man, because of the quality of feed and way in which they are fed, making exposure to moldy feed and possible toxins more frequent. Also, the foraging habits of some animals may expose them to mycotoxins more frequently than man.

In the absence of definitive data to interpret the role of mycotoxins in human disease, one must turn to less direct data such as animal studies, epidemiological data and reports of isolated incidences of human disease, thought to be related to mycotoxins, but for which complete proof is lacking. Based on these considerations, the types of diseases that may be caused in man by mycotoxins can be divided into acute toxicity, chronic toxicity and/or carcinogenicity. The greatest amount of information available in this regard concerns aflatoxins.

**Acute toxicity**

There are reports in the literature that associate aflatoxins with acute poisonings in humans. These reports all involve children and have been reviewed by Moss (112) and Shank (146). In one report, 26 persons in two Taiwan farming villages suffered illness (146). The ill persons were in three families of 10 households. Five households had consumed moldy rice for periods up to 3 weeks and experienced the intoxication. Members of the same families but living in different households which consumed rice that was not moldy were not affected. There were three deaths, all children, ages 4, 5 and 6 years. Symptoms of the disease were edema of the lower extremities, abdominal pain, vomiting, palpable liver but no fever. Samples of the rice were assayed for aflatoxin B₁, which was found in two samples at levels of about 200 µg/kg. Another suspected case of fatal aflatoxin poisoning occurred in Uganda and involved a 15-year-old-boy (145). Upon admission to a hospital, the boy had a history of abdominal pain, edema of the legs, palpable tender liver, and no fever, symptoms very similar to the Taiwan cases. Pathological findings included pulmonary edema, flabby heart, necrosis of the liver and fatty liver. The diet consisted mainly of cassava which was found to be moldy and contaminated with 1.7 mg of aflatoxin/kg. Extrapolation of data obtained with monkeys, and calculations of the amount of cassava consumed by the boy over a period of several weeks revealed that a fatal dose could easily have been consumed. Two younger children in the same family were ill with similar symptoms but recovered. It was postulated that because the young children ate less, they might have been exposed to sub-lethal dose levels. Studies in Thailand show that Reye's syndrome occurs in epidemic proportions in northeast Thailand (11). Reye's syndrome occurs in children and is characterized by vomiting, hypoglycemia, convulsions, coma and usually death. Pathological examination reveals severe cerebral edema and fatty degeneration of the liver, kidneys and heart. In one fatal case involving a 3-year-old-boy in northeast Thailand, it was found that the boy had eaten only leftover boiled rice for 2 days before becoming ill (11). Examination of the rice revealed that it was moldy and contained more than 10 mg of total aflatoxins/kg. Autopsy of Reye's syndrome cases in Thailand has shown aflatoxin B₁ in human tissue specimens from 22 of 23 cases, and in two cases the toxin levels in the tissues were similar to those in tissues of monkeys that had been given an approximate LD₅₀ dose. Studies of infantile cirrhosis of the liver in India showed that of 16 mothers with children suffering from cirrhosis, four were secreting a
compound in the milk that resembled aflatoxin B₁ on thin-layer chromatographic analysis, and one was secreting aflatoxin M₁. Aflatoxin B₁ was found in the urine of the cirrhotic children (112).

These specific suspected cases of aflatoxin poisonings in humans exhibit striking similarities to acute effects of aflatoxicosis in animals. The involvement of children suggests that young humans may be more susceptible than older individuals, which is the case with most animal species. Symptoms, pathological changes and dose levels seem to be in agreement with data reported for animals, particularly primates.

Carcinogenicity and chronic toxicity

The incidence of primary liver cancer varies throughout the world with highest incidences occurring in the tropical regions of Africa, south of the Sahara, India, Southeast Asia, Japan and the Philippines (83,146). In Uganda where the incidence of liver cancer is high, food crops are also found to be highly contaminated with aflatoxins. In addition, there is a geographical distribution of aflatoxin contamination of food in Uganda that corresponds to the geographical incidence of liver cancer within the country (3). The same type of correlation between aflatoxin contamination of peanuts and liver cancer was found in Swaziland (146). Studies in Kenya from 1967 to 1970 correlated the incidence of liver cancer and aflatoxin contamination of food supplies with altitude. As altitude increased and temperatures became more moderate, the levels of aflatoxin in the food decreased, as did the incidence of liver cancer (126). Other epidemiological studies by Shank et al. (147-151) in Thailand found similar correlations between dietary aflatoxin and incidence of liver cancer in different geographic regions of the country. Leftover foods were thought to be an appreciable source of aflatoxins in the diet.

Recently it has been suggested that chronic exposure to ochratoxin A in humans may be the cause of a fatal disease condition known as Balkan Endemic Nephropathy (50,85). This disease is manifested by renal disorders and occurs in certain areas of Bulgaria, Rumania and Yugoslavia. Balkan Endemic Nephropathy in humans bears striking similarity to porcine nephropathy in swine. Since ochratoxin A has caused kidney damage in almost all the animal species tested, Krogh (85) states that it is likely that the human kidney would also be similarly affected. In addition, recent studies have shown that ochratoxin A was found more frequently in foodstuffs from areas of Balkan Endemic Nephropathy than from non-endemic areas (89). Because of this, it has been suggested that human exposure to ochratoxin A is higher in the endemic areas, further suggesting possible involvement of ochratoxin in the etiology of Balkan Endemic Nephropathy (85).

Effect of diet and nutrition

Nutritional status of individuals exposed to aflatoxins may have a bearing on response to the toxin. This is suggested by the fact that malnutrition, aflatoxins and high incidences of liver disease, including liver cancer, are often all found in the same population. Madhavan et al. (107) showed that reduced protein intake in monkeys significantly increased their susceptibility to aflatoxins. Similar observations have been made with rats by Newberne et al. (119,120) and Madhavan and Gopalan (106). Thus, the greatest threat to human health is likely to be found in those countries least able to reject low quality foods and with the poorest conditions for adequate storage to prevent the development of aflatoxin.

IMPLICATIONS TO FOOD SAFETY

Mycotoxins can enter the food supply in several ways, but these can be grouped into two general routes of contamination, direct or indirect contamination (75). Direct contamination occurs as the result of mold growth on the food material itself. Almost all foods are susceptible to mold growth during some stage of production, processing, storage or transport. Mold growth on foods that are to be consumed directly can result in direct exposure to mycotoxins. Normally, foods in which evidence of mold growth has occurred are rejected by most persons. However, in certain regions, because of shortages, it sometimes becomes necessary to consume food of poor quality to avert starvation. In these situations, exposure to mycotoxins by consumption of food can occur. In some areas of the world, it is common practice to consume moldy foods either because some mold is almost always present, such as in tropical areas, or because of the common use of molds in the fermentation and preparation of foods, such as tempeh, soy sauce and other oriental foods. The molds used in the commercial production of these foods have been examined and shown to be non-mycotoxin producers (71,113,114). However, in these areas of the world, home production of mold-fermented foods is also practiced. It is very possible that these home fermentations may become contaminated with mycotoxin producing molds and thus be a direct source of mycotoxins in the food (71). Direct contamination of and exposure to mycotoxins in food appear to be more of a hazard in tropical areas and regions where food shortages exist and where there is less aversion on the part of the population to consuming moldy food.

Indirect contamination of food occurs as the result of using a food ingredient contaminated with mycotoxins. Processed and prepared foods are the types of foods involved in indirect contamination. Indirect exposure to mycotoxins can also result from consumption of animal products which contain mycotoxin residues where the food producing animal has consumed moldy feed. Indirect routes of contamination are more of a problem
in those areas of the world where food is more highly processed such as the U.S., Canada and Europe. The recorded incidences of indirect contamination of foods are lower than those of direct contamination.

**Nuts and oilseeds**

Aflatoxins have been found in a number of nuts, nut products and oilseeds. Peanuts, Brazil nuts, pecans, pistachio nuts and cottonseed have been shown to be susceptible to contamination (156). Contamination of peanuts occurs primarily in the field during harvest, when the seeds are being dried. Mechanical damage, insect damage and excessive rain during the drying period are all contributing factors to invasion by and growth of *A. flavus*. Since most aflatoxin contamination occurs during the drying period, it is possible to screen nuts for processing and divert contaminated lots from edible use. With peanuts, it is even possible to divert individually contaminated seeds if the lot is not heavily contaminated. Sorting occurs at the farmer-buyer level and again at the sheller-processor level (61,80,156). Studies have shown that peanuts consumed as roasted in-shell nuts in the U.S. are relatively free of aflatoxins at the consumer level. This is due to a variety of reasons, including inspection and sorting that occurs before marketing (156). In addition, processed consumer peanut products in the U.S. have also been found to be relatively free of aflatoxins. Again, this is due to a number of reasons, but inspection, sorting and roasting all contribute to substantial reduction of aflatoxins in processed peanut products at the consumer level (156). Most of the credit for this low level of aflatoxin in consumer peanut products in the U.S. goes to the extensive cooperation between industry and government regulatory agencies in efforts to control the problem and prevent exposure of the consumer to aflatoxin. It has been suggested that because of this awareness and cooperation, the American consumer is now getting safer, higher quality peanuts and peanut products than ever before (61). This is in sharp contrast to conditions in other countries, such as in Southeast Asia and Africa, where studies have shown contamination of peanuts and peanut butter with aflatoxin has ranged from 17 to 97% of samples with levels of 213 to 1530 µg/kg encountered (156). Aflatoxin contamination of Brazil nuts, pistachio nuts and other nut products at the consumer level in the U.S. has similarly been reduced and controlled by inspection at importation ports and programs of education and certification worked out with importers and producing countries (156).

Aflatoxin contamination of cottonseed is also a problem in the U.S. and worldwide. Most of this contamination occurs in the field and can result in contaminated meal being used as feed (156). This may result in significant aflatoxin residues in certain animal products, particularly milk. Treatment with ammonia appears to be a potential means of detoxifying aflatoxins in oilseed meals, making them safe for animal feeds (62). Food oils from peanut, cottonseed and copra may be contaminated with aflatoxin. Normally, the refining process for these oils, as used in the U.S., removes aflatoxin. However, in countries where unrefined oils are used, these can be a source of human exposure to aflatoxins (62,156).

**Grains**

Grains, especially corn, are also subject to mycotoxin contamination. Contamination may occur in the field and during harvest and storage. Field fungi include *Fusarium* species, and contamination with these organisms can occur if certain high moisture conditions exist. It is also thought that invasion by *A. flavus* and aflatoxin production can occur in the field as well as during storage (70). Again, damage to the seed coat from insects, hail or mechanical handling and moisture content are primary factors which permit invasion and growth by *A. flavus*. It is thought possible that the second generation European corn borer may feed on the kernels and may play a role in damaging the corn and disseminating the fungus (104). Also, *Fusarium* species can infect corn in the field through the silks and there is experimental evidence that *A. flavus* can also invade corn in this way (70,157). It is now thought that most aflatoxin contamination of corn in the U.S. occurs before harvest (157).

With newer methods of mechanical harvesting, using picker-shellers at higher moisture contents of corn, there are increased chances for mechanical damage. Since corn harvested by picker-sheller must be mechanically dried, any delay in the drying process can result in mold growth. Such delays can occur as the result of overtaxing of drying equipment during peak harvest time, fuel shortages or attempts to conserve fuel and dry at lower temperatures. In some instances at peak harvest time, drying equipment and storage facilities are overtaxed to the extent that grain may temporarily be stored on the ground. In these situations, conditions favorable to mold growth may occur. Remoistening of grain during storage and transport either accidentally by leaking structures, condensation, or by “sweating” of cold grain may cause moisture levels in dry grain to rise to sufficient levels to permit mold growth. Even if only a small portion of the grain becomes moldy, very high levels of aflatoxin can be produced in a few kernels. These few kernels may contain enough aflatoxin to contaminate an entire lot with unacceptable levels of aflatoxins when the corn is milled into meal or feed.

Aflatoxin contamination of corn is a worldwide problem and appears to be most severe in the Philippines, Thailand and Uganda, where the incidence of aflatoxin contamination ranges from 35 to 97% of the corn supply (157). In the U.S., corn from the southeastern U.S. is more susceptible to contamination (41% incidence level) than corn from the midwest (2.5% incidence level). Aflatoxin levels in corn from the Southeast average 18 µg/kg, whereas contamination levels in the Corn Belt average less than 1 µg/kg (157). In some instances, processing of corn into food or feed
products may reduce the aflatoxin level. For example, alkali processing of corn for preparation of masa used in tortillas substantially reduces aflatoxin content (156, 157, 169). Wet and dry milling of corn tends to move the aflatoxin concentration to the feed grade and oil fractions (156). Other grains such as wheat, barley, rye, oats, grain sorghum, millet and rice appear to be less susceptible to aflatoxin contamination than corn, provided they are properly stored and handled (157).

Fruits and vegetables

Molds are frequent contaminants of fresh fruits and vegetables. Apples are susceptible to a rot caused by *P. expansum*. This organism produces patulin, and rotted apples have been shown to contain patulin (12, 155). Patulin has also been found in commercial apple juice (143, 177). Apples used for juice and apple butter are sometimes of poorer quality than apples used for direct consumption (76). If a few such apples contain patulin, entire lots of juice or other products may contain patulin. Sorting of apples before processing to eliminate rotted apples is essential to preventing contamination with patulin.

Low levels of aflatoxin contamination have been detected in figs (14). In surveys of dried dates and raisins, no aflatoxin has been found, though *A. flavus* may be a common contaminant of these commodities (156). Only limited incidences of aflatoxin in wines have been detected, with the average aflatoxin content being lower than 1 μg/l (156). In experimental studies with lettuce, cauliflower and celery and *A. parasiticus*, Raghu et al. (134) found that fungus did not grow well on these substrates and that no detectable levels of aflatoxin were found.

Animal products

Contamination of foods of animal origin with mycotoxins is a possible concern. Aflatoxin *M₁* has been found in the milk of lactating animals fed aflatoxins. Aflatoxins have been found in commercial fluid milk in the U.S., Germany and South Africa, and in commercial samples of nonfat dry milk and cottage cheese in the U.S., and in various imported cheeses (156, 170). Most of the aflatoxin found was in the *M₁* form and probably came from the feeding of aflatoxin-contaminated feed. Aflatoxins have also been found in livers, kidneys and other tissues of pigs in feeding trials with aflatoxin-contaminated diets (76). Levels were highest in livers and kidneys with only trace amounts occurring in heart, muscle and adipose tissues. Aflatoxins have also been found in the tissues of broiler chickens and eggs of laying hens given experimentally contaminated feed (157). In these animal products, aflatoxins have been found both as *B₂* and *M₁*. While the levels of aflatoxins in these foods are generally low, these studies indicate that indirect exposure to aflatoxins could occur from consumption of products of animal origin if the animals were fed aflatoxin-contaminated feed.

Residues of ochratoxin A have also been found in animal tissues including liver, kidney, muscle and adipose tissues (76, 85). These were tissues of bacon pigs (84) and poultry (51) in Denmark. Ochratoxin has also been found in pork meat in Yugoslavia (89). Thus, human exposure to ochratoxin might also occur as the result of the consumption of animal products from animals fed ochratoxin-contaminated feed.

Refrigerated foods

Mold growth on foods, such as cheeses and cured meats, stored at low temperatures is a common and recurring problem (57). Certain molds are known to be capable of producing mycotoxins at temperatures as low as -2 to 10 C. Many of these molds belong to the genus *Penicillium* which are capable of growth over a wide range of temperatures, including temperatures commonly employed in household refrigerators and supermarket display cases (135). Some psychrotrophic molds may grow at temperatures below 0 C (121). Recently Torrey and Marth (166) reported isolation of potentially toxic molds from home refrigerators and foods stored in home refrigerators.

Kuehn and Gunderson (92) reported that fungi associated with frozen fruit-filled pastries were capable of growing at 0 and 5 C. These workers also found that 50% of the psychrotrophic flora associated with frozen pastry products were members of the genus *Penicillium* (93). Several *Penicillium* species isolated by Kuehn and Gunderson (93) are now known to be potential mycotoxin-producing organisms (Table 1). These organisms include *P. cyclopium*, *P. expansum*, *P. frequentans*, *P. martensii*, *P. puberulum*, *P. urticae*, and *P. viridicatum*. Known mycotoxins produced include penicillic acid, patulin and ochratoxin A (39, 144, 172).

In a more recent study, 82% of the molds found on refrigerated Cheddar cheese belonged to the genus *Penicillium*, 7% were *Aspergillus* species and 1% were *Fusarium* species (26). Toxicological screening of molds isolated from Cheddar cheese indicated that 19.8% of the isolates were toxic to chicken embryos, causing 50% mortality or more. Thin layer chromatographic examination of chloroform extracts of the mold cultures showed the presence of known mycotoxins, including patulin, penicillic acid, ochratoxin A and aflatoxins, in 7.2% of the culture extracts (26). In further studies, most molds found on Swiss cheese were found to be *Penicillium* species which were capable of growing at 5 C (17). Toxicological screening of these isolates showed that 32% of culture extracts of the molds were toxic to chicken embryos. Known mycotoxins found were ochratoxin A, penicillic acid, patulin, citrinin and aflatoxin. In a survey of domestic and imported cheeses, Bullerman (19) reported that the predominant organisms found were *Penicillium* species and that toxigenic species of *P. cyclopium* and *P. viridicatum*, as well as *A. flavus*, were commonly found.

Several workers have studied the formation of mycotoxins in artificially inoculated cheeses and meats. Lie and Marth (98) showed that *A. flavus* and *A. parasiticus* would grow and produce substantial quan-
tities of aflatoxins on Cheddar cheese at room temperature. Oldham et al. (122) working with *A. flavus* and Cheddar cheese obtained low levels of aflatoxins at 25 C but none at 4.4 or 7.2 C. Kiermeier and Gross (82) obtained aflatoxin production on Tilsit cheese but not on Camembert and Romadur cheeses. Other workers have reported aflatoxin production in Tilsit and Emmental cheeses (55,139). Shih and Marth (152) reported production of aflatoxins on brick cheese at 12.8 C by *A. parasiticus* after 1 week of incubation, and at 23.9 C by *A. parasiticus* after 1 week and by *A. flavus* after 14 weeks of incubation. It is generally believed that *A. flavus* and *A. parasiticus* will not grow and produce aflatoxins at temperatures below 13 C (76). However, van Walbeek et al. (171) reported that a strain of *A. flavus* produced aflatoxin at 7.5 and 10 C in 4 weeks. Strain differences might account for aflatoxin production at temperatures approaching refrigerated storage, but, for the most part, aflatoxins are not considered to be a problem if foods are kept under adequate refrigeration. This is, however, a key point since temperatures in refrigerators and refrigerated display cases may vary considerably. van Walbeek et al. (171) reported in a survey of domestic refrigerators, that the minimum temperatures ranged from 0 to 10 C. More recently, Torrey and Marth (167) reported that mean temperatures ranged from 3.9 to 11.9 C in two home refrigerators with the total range being 1.7 to 20.2 C. In that study an aflatoxigenic strain of *A. parasiticus* did not grow at 8 C over a period of 21 days; however, *Penicillium* species grew at 5 C. Recently, Kiermeier and Behringer (81) reported aflatoxin formation in moistened milk powder at temperatures between 1 and 5 C and at 10 C. On the other hand, Lieu and Bullerman (99) reported that *A. flavus* did not grow on any of several food substrates at 5 or 12 C, but grew extensively and produced aflatoxins at 25 C.

Stott and Bullerman (158) inoculated Cheddar cheese with a patulin-producing strain of *P. patulum*, and found that the mold grew extensively on the cheese at 5 and 25 C. Patulin production on the cheese was not observed at 5 C and only small variable amounts of patulin were found at 25 C. In the samples that contained patulin, the toxin was localized in the mold mycelia and the first 3-mm layer of cheese. Lieu and Bullerman (99) observed similar results with patulin- and penicillic acid-producing organisms grown on Swiss and Mozarella cheeses. In this study the *Penicillium* species grew extensively on the cheese at 5, 12 and 25 C; however, no patulin or penicillic acid were detected in the cheese as a result of the mold growth. Olivigni and Bullerman (123) working with an atypical isolate of *P. roqueforti* obtained patulin and penicillic acid production at 5, 12 and 25 C on laboratory media, but the toxins were not produced on Cheddar or Swiss cheeses.

In addition to refrigerated cheeses and frozen pastries, several workers have reported the incidence of toxic molds in cured and smoked meat products. Bullerman and Ayres (21) screened 66 *Aspergillus* and *Penicillium* isolates from country-cured hams and fermented sausages for aflatoxins and found aflatoxin production in one strain of *A. flavus* (NRRL A16-100). Strzelecki and Badura (162) isolated *A. flavus* and *A. versicolor* from dry Cracower sausage in Poland. Four strains of *A. flavus* out of 36 total isolates were capable of aflatoxin production in yeast-extract sucrose broth. Of 562 molds isolated from country-cured hams by Sutic et al. (164), 403 were *Penicillium* species and 121 were *Aspergillus* species including toxigenic strains of *A. flavus* and *A. versicolor*. In a study of European dry salami, Ciegler et al. (42) isolated 346 cultures of *Penicillium* species. About 10% of the *Penicillium* cultures including six species were capable of producing penicillic acid in liquid media. Leistner and Ayres (96) isolated 307 molds from 40 samples of country cured hams and 27 fermented sausages. *Aspergillus* and *Penicillium* species predominated on country-cured hams and *Penicillium* and *Scopulariopsis* predominated on fermented sausages. Wu et al. (182) screened 89 cultures of *Aspergillus* and 54 cultures of *Penicillium* isolated from aged cured meats for toxicity to chicken embryos and found about 16% of the aspergilli were toxic and about 1% of the penicillia were toxic. Known mycotoxins were not detected. Bullerman (20) isolated potentially toxic strains of *P. cyclopium, P. viridicatum* and *A. flavus* from domestic and imported cured meats. In that study *Penicillium* species were predominantly found.

Experimentally, aflatoxin production on cured meats has been reported at temperatures as low as 15 C. Bullerman et al. (23,24) observed aflatoxin production on bacon, ham and aged salami at 15 C. Tauchman (165) likewise observed aflatoxin production on dry sausage at 15 C. However, Oldham et al. (122) working with cured luncheon meat did not observe aflatoxin production at 4.4 and 7.2 C. Likewise, Lieu and Bullerman (99) did not observe aflatoxin production by *A. flavus* on bologna or bacon at 5 and 12 C; however, aflatoxin production was evident on these substrates at 25 C.

Wu et al. (181) showed that seven strains of *P. viridicatum* isolated from country-cured ham were capable of producing citrinin on this type of ham when grown at temperatures from 15 to 30 C. Ochratoxin-producing strains of *P. viridicatum* have also been isolated from mold fermented sausages (108). Halls and Ayres (64) reported that strains of *A. versicolor* produced sterigmatocystin on country cured ham at 20 and 28 C. In studies with patulin- and penicillic acid-producing organisms grown on meats, production of these toxins on meats was not observed at 5, 12, or 25 C even though extensive growth of the molds occurred at all temperatures (42,99,123).

It has been suggested that the lack of production of patulin and penicillic acid on cheese and meat substrates may be because these substrates are low in carbohydrate and high in protein (42,99,123,159). This is supported by
the fact that other natural substrates high in protein, such as soybeans, peanuts and cottonseed, likewise do not support penicillic acid production (41). In addition, other studies have shown that laboratory media which are lacking in carbohydrate but high in protein support extensive growth of patulin- and penicillic acid-producing molds, but little or no production of either toxin (123, 159). Incubation temperature is not the determining factor controlling patulin or penicillic acid production in the case of Penicillium species, since these organisms produced the toxins in suitable substrates, including potato dextrose broth, yeast-extract sucrose broth, shredded wheat, and cooked cornmeal, at 5, 12 and 25 C (99, 123, 159). Low available water in the cheese and meat substrates would probably adversely affect production of patulin and penicillic acid in these substrates; however, this does not adequately explain the extensive growth but lack of production of these toxins in broth substrates containing no carbohydrates, but high amounts of protein (123, 159).

Stability of mycotoxins in foods

Once mycotoxins have contaminated and become a part of a food system, it is important to know how long the toxins will persist and retain biological activity. Aflatoxins are generally thought to be stable in most food products. Thermal processing of food, other than roasting of nuts, is not likely to reduce the aflatoxin content, whereas roasting may cause a reduction of 40 to 60% of the aflatoxin present (157). Alkaline treatments and refining of food oils either destroy or remove aflatoxins (157). Aflatoxin levels have been reported to decline in raw peanut butter in storage for up to 6 months (174). Strzelecki (161) reported that recovery of aflatoxin from meat decreased with increasing storage time. Murthy et al. (115) observed a decrease of 98 to 80% in recovery of aflatoxin B1 from fresh beef stored at -18 C for 183 days. Chu et al. (34) found that aflatoxin B1 was partially lost in the mashing and brewing process. Dam et al. (44) reported that fermentation of grains experimentally contaminated with aflatoxin caused a loss of about 60% of the added aflatoxin and that further treatment involved in isolation of distillers protein concentrate destroyed total aflatoxins in excess of 90%. In studies with peanut meal and peanut butter, Bauer (9) reported no significant changes in aflatoxin levels when the products were held at 23 C and 50% relative humidity exposed to air for 2 years. Lieu and Bullerman (99) found that aflatoxins B1 and G1 were essentially stable in Swiss cheese, bologna and cooked cornmeal for up to one week at 5 C. Recoveries of aflatoxins ranged from 90 to 100%.

Patulin and penicillic acid appear to be less stable than aflatoxins in certain foods. Scott and Sommers (142) found that patulin and penicillic acid were both stable in grape and apple juice but not in orange juice and flour. They attributed the instability in orange juice and flour to the presence of thiol compounds. Pohland and Allen (127) also reported that patulin was stable in apple juice and dry corn but unstable in wet corn, Durum wheat and sorghum. Hofmann et al. (72) and Ciegler et al. (42) investigated potential production of patulin and penicillic acid in meat and meat products. They concluded that both patulin and penicillic acid at the pH of meat and meat products would react with sulfhydryl compounds and amino acids normally occurring in meat and therefore would not be detected by chemical assay procedures. Scott and Bullerman (160) found that patulin became undetectable when added to Cheddar cheese and stored at 5 and 25 C for various periods of time. Further, Lieu and Bullerman (99) found that patulin and penicillic acid became undetectable in bologna after 12 and 48 h, respectively, when stored at 5 C. With Swiss cheese a low level of patulin and penicillic acid persisted, with 5 to 8% of the added toxins being detected after one week at 5 C. In these same studies, patulin and penicillic acid were more stable in cooked cornmeal with about 80% of the toxins recoverable after one week at 5 C. Patulin has also been reported to disappear from apple juice undergoing fermentation by Saccharomyces cerevisiae and Saccharomyces ellipsoideus (68).

The instability of patulin and penicillic acid in foods such as meat and cheese has been attributed to combination of the toxins with amino acids and compounds containing sulfhydryl groups (42, 72, 100, 127, 142). Binding of patulin and penicillic acid to sulfhydryl groups has been shown to reduce the biological activity of these toxins. Hofmann et al. (72) found that patulin bound to glutathione was no longer toxic to chicken embryos, mice and rabbit skin. S-alkylated adducts formed by combination of penicillic acid with cysteine or glutathione were found to be nontoxic to mice and quail by Ciegler et al. (42). In the same study, it was shown by chicken embryo tests that the penicillic acid adduct of glutathione was about 40 to 50% as toxic as penicillic acid and the penicillic acid adduct of cysteine was reported to be as toxic as penicillic acid itself. More recently, Ciegler et al. (38) showed that the adduct of patulin and cysteine was nontoxic to mice and chicken embryos but remained teratogenic to chicken embryos. Lieu and Bullerman (100) studied the toxicity of the adducts of combinations of patulin and penicillic acid with glutathione and cysteine and found them to be non-inhibitory to Bacillus subtilis at 50(pg of toxin equivalent. Patulin and penicillic acid adducts of cysteine at 50 and 150 pg of toxin equivalent were nontoxic to brine shrimp larvae, and the patulin-glutathione adduct and patulin-cysteine adduct both at 100 pg of patulin equivalent were not toxic to 4-day-old chicken embryos. However, the penicillic acid adduct of cysteine at 100 pg equivalent of penicillic acid possessed considerable toxicity to embryos, but the penicillic acid-glutathione adduct in the same amount was less toxic. Simulated peptic digestion of S-alkylated adducts of the two toxins did not result in the regeneration of free toxins. No teratogenic effects were observed in this study.
Ochratoxin appears to be more stable in foods than patulin or penicillic acid but probably somewhat less stable than aflatoxins. In one study, Harwig et al. (66) studied the effect of canning dried white beans contaminated with ochratoxin A. Thermal processing at 121 C for 1 h reduced the ochratoxin content by 11%, whereas processing at the same temperature for 4 h reduced ochratoxin content by 34%. Soaking and blanching resulted in losses of 21% and 10% of ochratoxin, respectively. In another study, autoclaving of oatmeal and rice cereals for 3 h reduced the amount of added ochratoxin by 60% (169). Simulated coffee roasting conditions, where temperatures of 200 F for 5 min are reached, were found to destroy ochratoxin (97). Ochratoxin and citrinin were also found to be destroyed by the malting and brewing process if moderately contaminated barley was used (34,87). Highly contaminated barley retained 2 to 7% of the original amount of ochratoxin, but barley in that condition was severely deteriorated and would not likely be accepted for brewing use.

From these studies it would appear that of the mycotoxins of concern in foods, aflatoxin would be expected to be the most stable in all foods. Ochratoxin A would also be expected to be stable in many foods, whereas the stability of patulin and penicillic acid would be quite dependent upon the nature of the foodstuffs in which they are found. Stability of patulin and penicillic acid would be expected to be greatest in dry grains and apple products and least in high protein foods such as cured meat and cheese. More studies on the stability of these and other mycotoxins in foods are needed to fully assess the long term stability of all mycotoxins in foods.

Regulation and control of mycotoxins

Control of mycotoxins in foods is a complex and difficult task. Information regarding toxicity, carcinogenicity and teratogenicity to humans, extent of contamination and stability of mycotoxins in foods is lacking for most mycotoxins. Such information is necessary to establish regulatory guidelines, tolerances and seizure policies. In the United States, the U.S. Food, Drug and Cosmetic Act defines food as adulterated if it contains "any poisonous or deleterious substance which may render it injurious to health" (138). The act also gives the Food and Drug Administration (FDA) authority to enforce the Act and remove from interstate commerce any food or feed found to be adulterated with such substances. The FDA treats aflatoxins as poisonous and deleterious substances, and regulates them accordingly. The law distinguishes between poisonous substances that are natural components of foods and those which are added to foods. Aflatoxins, though of natural origin, are not considered natural components of foods and therefore are treated as added components, though as unavoidable contaminants (53,138). Aflatoxins, while they are known carcinogens, are not regulated under the Delaney Amendment since they are considered to be unintentional additives. The Delaney Amendment applies only to those food additives that are intentionally added for some specific purpose of preservation or processing. In actuality there is no tolerance level for aflatoxins in any food (176), since toxicological data for mycotoxins, including aflatoxins, upon which a safe tolerance level might be based are lacking. Thus, a safe tolerance level for any of these compounds has not been established. In the absence of tolerances, FDA has set what it considers to be practical limits for aflatoxins in foods and feeds, based primarily on the limits of detections and measurement of analytical methods and to some extent on the ability of agronomic and technological practices to prevent contamination (76). Practical limits have been set forth by FDA as "working guidelines" for regulatory action and apply to all products known to be susceptible to aflatoxin contamination, and include animal feeds (138). In the U.S., the Food and Drug Administration has a working guideline of 20 ppb (µg/kg) for aflatoxins in susceptible commodities. Currently, FDA is proposing to lower the guideline to 15 ppb for peanut products (138). This change is being proposed because it is clear that present analytical methods can routinely measure aflatoxin levels below 20 ppb. Also, when specific information indicates that contamination of a finished food product could have been controlled or avoided by application of good manufacturing practices, the guideline is not applicable (176). In such situations FDA bases its decision on the specific facts of the case and may, in fact, resort to seizure and compliance action for amounts of aflatoxin less than the established guidelines. Such actions are considered justified and have been taken by FDA in the past (176).

On December 7, 1977, the Food and Drug Administration (53) announced the establishment of an "action level" for aflatoxin in whole milk, skim milk and low fat milk of 0.5 ppb. This means that FDA prohibits the shipment of milk in interstate commerce that contains more than 0.5 ppb of aflatoxin. The action was taken in response to the fact that adverse weather conditions and possible insect damage had resulted in a high degree of aflatoxin contamination of corn in the southeastern U.S. in 1977, and it appeared that aflatoxin-contaminated corn was being fed to dairy animals. The lower level for milk was imposed because milk containing aflatoxins may pose a special risk to infants and young children who may consume large quantities of milk.

Most regulatory considerations to date concerning mycotoxins have involved aflatoxins. However, FDA is currently studying toxicological data and contamination incidences of foods for other mycotoxins including ochratoxin A, patulin, penicillic acid, sterigmatocystin, zearalenone and T-2 toxin. As sufficient toxicological data become available to permit an assessment of the significance of these toxins to human and animal health, guidelines and regulations for these toxins can also be expected (138).
A number of countries have established regulatory programs for aflatoxins in food and feeds. These include Brazil, Canada, Denmark, the United Kingdom, France, Hungary, India, Japan, the Netherlands, South Africa and the Federal Republic of Germany in addition to the U.S. (56,137). The regulations contain guidelines for aflatoxin contamination of human food in the range of 5-30 ppb (137). The Food and Agriculture Organization (FAO) of the United Nations has sought to assist developing countries in establishing effective control systems and regulations on aflatoxin contamination of foods in these areas of the world (69).

While the efforts of regulatory agencies such as FDA are very important in preventing human and animal exposure to mycotoxins, the responsibility for actual control and prevention of this hazard lies with the agricultural and food industries. It is at this level that control measures to prevent contamination and eliminate contaminated commodities from the food supply must be applied. In addition to FDA, the U.S. Department of Agriculture (USDA) is also involved in mycotoxin control in certain commodities such as peanuts and grains. The USDA has a testing program for peanuts beginning at the farm level and continuing through the marketing system. Also, joint industry-USDA-FDA programs are in effect to control aflatoxin contamination of agricultural commodities such as peanuts, cottonseed, copra, corn and animal feeds (176). These relationships and voluntary programs have resulted in improved industry and government communications and development of food processing and manufacturing practices that have helped to reduce and eliminate mycotoxin contamination of a number of commodities (176).

The control of mycotoxins in foods involves many factors. The best approach to eliminating mycotoxins from foods is to prevent mold growth. However, this is not always as simple and straightforward as it may seem. Mold growth must be prevented at all levels of production, harvesting, transporting and storage of foods. This involves prevention of insect damage and mechanical damage to agricultural commodities as well as moisture control at levels which do not permit mold growth and storing at temperatures and under conditions which minimize mold development. Control of mycotoxins also involves quality control procedures to detect and remove contaminated products from commercial channels before they reach the consumer.

CONCLUSIONS

Our appreciation of the significance of mycotoxins to food safety and human health continues to evolve and increase. While there is no direct evidence, in the form of controlled feeding experiments, for the involvement of mycotoxins in foodborne human disease, the indirect evidence is strong and continues to accumulate. In light of the numerous animal species found to be susceptible to the various effects of mycotoxins, it is difficult to believe that man would not be similarly affected.

Regional studies of acute and chronic disease patterns in humans, and the association of these with mycotoxin contamination of diets and foodstuffs in the same regions, are further evidence of the probable involvement of mycotoxins in foodborne human disease. This is particularly significant when symptoms, and toxin levels in body fluids and tissues, of human victims are found to be similar to those observed in test animals in controlled feeding experiments. It simply remains a matter of time until additional work proves the conclusive involvement of mycotoxins in human disease. Therefore, it is of utmost importance that every attempt be made to keep mycotoxins out of the food and feed supply. Not only must food-producing animals be protected from mycotoxins to maintain production, but also to prevent the occurrence of mycotoxin residues in human foods of animal origin. Foods and commodities must be stored under conditions which prevent mold growth on the farm, in storage facilities, in retail outlets and in homes. Thus, protection of the human food supply from contamination with mycotoxins must occur along the entire food chain, from the point of production on through to the consumers' own refrigerator and kitchen.

REFERENCES


SIGNIFICANCE OF MYCOTOXINS


A Field Topic

Effect of Disk Weight and Filtration Vacuum Level on Flow Time of Yellow Staining Milk Through Lintine Disks

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(Received for publication April 17, 1978)

ABSTRACT

About a pint (550 ml) of yellow staining milk was filtered using vacuum levels of 76 and 381 mm of Hg through Lintine sediment disks which weighed 65, 90, or 112 mg. The time taken for the milk to flow through the disks was longer with heavier disks and the lower vacuum level. The fat retained on the disks and the concentration of \( \beta \)-carotene in that fat increased with disk weight.

The filtration sediment test is used to assess the cleanliness of a milk supply (11). Sometimes during routine factory testing, milk samples pass through the disks slowly, or not at all, because of clogging (2.4.6). More often a yellow stain-like material, called yellow stain in New South Wales, is retained from the milk (3,4). The stain covers all of the disk filtering area and differs in appearance from the flecks and clots that are occasionally seen. Milk showing either or both these characteristics is considered abnormal (1,2,5).

Chumney and Kleyn (2) compared milk flow time through sediment disks as measured with the Milk Quality Gauge (MQG), and the Wisconsin Mastitis Test (WMT) score. They found that there was not a good correlation between the two. However, milk-flow time showed a better correlation with the presence of yellow stain on the disks. Explanations offered by them for the poor relationship between flow time and WMT score, were decrease in WMT score as the milk aged, and high sediment content.

However, disk weight, vacuum level used to draw the sample through the disks, and milk flow time influence the intensity of the yellow color left on disks (9). I report here an experiment showing the effect of disk weight and vacuum level on rate of milk flow through the disks. Because retained fat and \( \beta \)-carotene are known to be the source of the yellow stain (7,10), some data on fat retention and \( \beta \)-carotene concentrations in relation to disk weight are also presented.

MATERIALS AND METHODS

The milk used was from one lot of clean farm-refrigerated bulk milk which left a yellow stain on sediment disks. Lintine \( ^\circledR \) sediment disks (Filter Fabrics, Chicago) were sorted and weighed (9). The disks used (Table 1) were within ±1 mg of the stated weight. Since the diameter of the disks is constant, the weight is a measure of the thickness of the disks.

The sediment test was done as follows. Milk was divided into 550-ml portions in plastic bottles and heated to 40°C in a waterbath. Disks were placed in a hand sediment gun, orifice diameter 19.5 mm, set up over a Buchner flask attached to a vacuum source and fitted with a vacuum gauge. The contents of a plastic bottle were poured into the sediment gun and vacuum applied either at 76 or 381 mm of Hg according to the gauge. The time of flow was measured from the moment vacuum was applied until the last milk passed through the disk. The funnel of the sediment gun was released and excess liquid removed from the disk by applying full available vacuum. Time of flow for each disk weight/vacuum level combination was determined in duplicate. The disks were dried overnight on the bench and the fat and \( \beta \)-carotene determined as previously described (10).

RESULTS AND DISCUSSION

Results in Table 1 show that the time of flow varied with weight of the disks, i.e., thickness of disks, and vacuum level. Flow time was longer with the heavier disks, and with a lower vacuum level. The yellow stain became more intense as flow time increased.

Results show that small differences in disk weight critically affect flow time of yellow staining milk through Lintine disks. Differences in the vacuum level, i.e., the pressure applied across the disk to force the milk through, are also critical.

The increase in fat content on disks as disk weight increased would explain the clogging of disks and its subsequent effect on flow time. Retention of fat globules on Lintine disks is the cause of yellow stain (7,10). When yellow-stained disks are examined microscopically, small fat globules are seen spread over the fibers of the disks (8).

Results of analyses for \( \beta \)-carotene indicate that there is a selective build-up of this substance on the disks. The concentration in the milk

<table>
<thead>
<tr>
<th>Weight of disks (mg)</th>
<th>Fat on disks (mg)</th>
<th>( \beta )-carotene on disks (( \mu )g/g fat)</th>
<th>Time of flow (sec)</th>
<th>Vacuum level</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>4.4</td>
<td>17.4</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>90</td>
<td>6.2</td>
<td>20.9</td>
<td>165</td>
<td>12</td>
</tr>
<tr>
<td>112</td>
<td>28.7</td>
<td>49.6</td>
<td>NT(^b)</td>
<td>195</td>
</tr>
</tbody>
</table>

\(^a\)Results from disks using 381 mm of Hg vacuum. The \( \beta \)-carotene concentration in the milk was 9.4 \( \mu \)g/g fat.

\(^b\)Not tested.
was 9.4 μg/g of fat, but on the disks it was as high as 49.6 μg/g of fat. This is in agreement with a previous report (10).

If flow time through Lintine disks is to be used to assess whether a milk supply is abnormal, the variation in disk thickness, and the pressure differential used to force the milk through the disks, must be carefully controlled. It has been shown (9) that disks can vary in weight from 58 to 195 mg, indicating a wide variation in thickness. Thus randomly selected disks would be of no use for measuring flow time. I suggest that a satisfactory pressure differential would be that equivalent to 381 mm of Hg vacuum, i.e., a vacuum level of 15 inches of Hg.

O'TOOLE

ACKNOWLEDGMENT
I thank Mr. D. J. Firth for his assistance with chemical analyses.

REFERENCES

Report of the 3-A Sanitary Standards Symbol Administrative Council

There were two meetings of the Council held during this year; one took place in Denver, Colorado on October 25 & 26, 1977, and the second was held on June 6, 1978 in Chicago, Illinois.

Present members of the Council are: Dr. W. S. Clark, Jr., Chairman; Mr. P. K. Gitton, Vice-Chairman; Mr. E. O. Wright, Secretary-Treasurer; Mr. P. J. Dolan, Asst. Secretary-Treasurer; Mr. D. G. Colony, Dr. H. V. Atherton, Mr. O. M. Osten, Mr. D. D. Fry.

The Council is now up to full strength. Dr. Henry Atherton was approved by the I.A.M.F.E.S. to replace Dr. K. G. Weckel on the Council. Dr. George Muck resigned because of his work load in other areas and David Fry was appointed by D.I.C. to replace him on the Council.

Several non-compliance reports were considered by the Council during the year. The Council publishes a list of holders of 3-A Symbol Council authorizations every six months in the Journal of Food Protection. There were several new applications received and processed during the past year. Following are a few of the Council's activities during the year:

1. Reflecting a procedures change, the Council, when listing an authorization on the published list, will show the manufacturer of that equipment if other than the company holding the authorization.

O'TOOLE

ACKNOWLEDGMENT
I thank Mr. D. J. Firth for his assistance with chemical analyses.

REFERENCES

Coming Events

Aug. 13-17--WORKSHOP ON EDUCATIVE PROCESSES IN FOOD MICROBIOLOGY. Sponsored by the Joint American Society for Microbiology/Institute for Food Technologists Committee on Food Microbiology Education. Quadna Resort, Hill City, MN. Contact: E. A. Zottola, Dept. of Food Science, University of Minnesota, St. Paul, MN 55108.

Aug. 29-31--FOURTH INTERNATIONAL IUPAC SYMPOSIUM ON MYCOTOXINS AND PHYCOTOXINS. Co-sponsored by World Health Organization and Swiss Society for Analytical and Applied Chemistry, Lausanne, Switzerland. For participation and poster presentation, contact: Prof. P. Krogh, Dept. of Veterinary Microbiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 or Prof. D. Reymond, IUPAC, Case postale 88, 1814 La Tour de Pezil, Switzerland.

Sept. 18-20--WESTPACK. Convention Center, Anaheim, CA.

Bower Wins NAMA Award

The Arthur J. Nolan Public Health Award of the National Automatic Merchandising Association (NAMA) was presented to William F. Bower, Deputy Director of FDA's Division of Retail Food Protection, at the association's annual convention in Atlanta in October.

Named after the vending industry leader whose pioneering efforts led to the first Public Health Service Vending Ordinance and Code in 1957, the award is voted by the members of NAMA's Health-Industry Council (AMHIC).

Bower was a charter member of the Automatic Merchandising Health-Industry Council, representing the National Association of Sanitarians and was the author of Oregon's vending sanitation law and inspection programs.

The Nolan Award has been presented since its creation in 1966 to six other public health, industry and university leaders in the field of vending sanitation.

SDSU wins Judging Contest

South Dakota State University's Dairy Products Judging Team claimed "the cream" of judging contests by winning the 57th National Collegiate Dairy Products Evaluation Contest in Anaheim, Calif., Nov. 6. The SDSU contingent had earlier claimed the second largest prize in their specialty by topping the field of the Chicago Dairy Tech Midwest Regional Contest in October.

The top ten field included Mississippi State University in second place, followed by Kansas State and the University of Minnesota tied for third. Oregon State, Iowa State, the Universities of Missouri and Wyoming, California-Fresno and Ohio State University rounded out the top ten in team scoring.

Wisconsin Honors Two at Annual Meeting

Two persons were honored by the Wisconsin Association of Milk and Food Sanitarians and the Wisconsin Dairy Fieldmen's Association at their Annual Convention Sept. 13 and 14 at Fond du Lac.

Merle Emond was named Wisconsin "Sanitarian of the Year" and Deborah Escher was named recipient of the 1978 scholarship award.

"A Year of Change," was the program theme. Bill Turner of Minneapolis, MN, keynote speaker in "What You Do Is Up To You," stressed the importance of individual performance on the job.

"Solar Energy on the Dairy Farm," was a presentation made by Harlan Flene of Babson Brothers. "Mutagens in Cooked Foods," was presented by Dr. Michael Pariza of the Food Research Institute. Kathey Flene of Babson Brothers.

Weigold Retires From DFISA

George Weigold, director of special projects for the Dairy & Food Industries Supply Association (DFISA) since 1973, has retired. Weigold's responsibilities for DFISA included the collegiate dairy products judging contest, international contacts, liaison with the Department of Agriculture and Commerce, as well as special projects.

Weigold has also served as managing director of Dairy Science International since 1960. He will continue to serve in this position.

A graduate of the University of Connecticut, Weigold was plant superintendent for 20 years of his family-owned business, Torrington Creamery of Torrington, CT. He later was general manager of a by-products plant in Puerto Rico.

In addition to other activities and honors, Weigold was named in 1972 for the Distinguished Service Award of the American Dairy Science Association.

Alberta Association Holds Annual Meeting in Calgary

The Alberta Association of Milk, Food, and Environmental Sanitarians held their Annual Meeting in Calgary on Sept. 28, 1978. The program included a tour of the Lucerne Meat Cutting Plant with a discussion of plant operations by Harvey Dietrich, and technical sessions featuring Dr. Harry Jackson and Dr. Barry Ruck. Dr. Jackson, chairman of the University of Alberta Food Science Department, offered a practical view of the meat system from farm to the consumer. Dr. Ruck, of the W. E. Greer Ltd., stressed the importance of developing a total sanitation system in a plant.

Walter Charles was named "Sanitarian of the Year." Charles recently retired from the Health Protection Branch in Edmonton.
Coming Events

Jan. 9, 11--FOOD PROCESSING SANITATION WORKSHOP. Sponsored by University of California Cooperative Extension. Jan. 9 session will be held at the Holiday Inn, Park Center Plaza, 181 Almaden Blvd., San Jose, CA. Jan. 11 session will be at the Proud Bird Restaurant, 11022 Aviation Blvd., Los Angeles, CA. Contact: Paulette De Jong, Food Science and Technology Dept., Univ. of California, Davis, CA 95616, 916-752-1478.

Jan. 10-11--DAIRY PROCESSORS CONFERENCE. Sponsored by Food Science Dept., University of Wisconsin-Madison. Sheraton Inn, Madison, WI. Contact: Myron Dean, Dept. of Food Science, Babcock Hall, 1605 Linden Drive, Madison, WI 53706.


Feb. 14-15--DAIRY AND FOOD INDUSTRY CONFERENCE. Ohio State University, Contact: J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., Ohio State University, Columbus, OH 43210.

Feb. 19-22--ICE CREAM TECHNOLOGY SHORT COURSE. Ramada-Crabtree Inn, 3926 Arrow Drive, Raleigh, NC 27612. Contact: W. S. Arbuckle or R. B. Redfern, Ice Cream Technology, 3413 Blue Ridge Rd., Raleigh, NC 27612.


Feb. 27-28--KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC., Annual Meeting. Contact: Dale Marcum, Milk Control Branch, Frankfort, KY.

Feb. 27-Mar. 1--THIRD DOMESTIC WATER QUALITY SYMPOSIUM. Sponsored by American Society of Agricultural Engineers and 17 national co-sponsoring organizations and agencies. St. Louis, MO. Contact: American Society of Agricultural Engineers, P.O. Box 410, St. Joseph, MI 49085, 616-429-0300.

Feb. 27-Mar. 2--TECH EX '79-ANNUAL WORLD FAIR FOR TECHNOLOGY EXCHANGE. Atlanta, GA. Contact: E. B. Prine, Vice President, Tech Ex '79, Dr. Dworkowitz & Assoc., P.O. Box 1748, Ormond Beach, FL 32074.


Mar. 6-7--VIRGINIA ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Donelson Brown Center for Continuing Education, Virginia Polytechnic Institute and State University, Blacksburg, VA. Contact: Marshall Cooper, 116 Reservoir St., Harrisonburg, VA 22801.

Mar. 9-10--SEVENTH ANNUAL FOOD INDUSTRY ASSOCIATION OF SOUTH CAROLINA CONFERENCE. Hilton Head, SC. Contact: T. C. Titus, Food Industry Association of SC, Box 708, Clemson, SC 29631.

Mar. 19-21--AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE ANNUAL TRAINING SCHOOL AND JUDGING CONTEST. Hilton Inn, Columbus, OH. Contact: C. Bronson Lane, AC DPI, P.O. Box 7813, Orlando, FL 32854.

Mar. 19-23--MID-WEST WORKSHOP IN MILK AND FOOD SANITATION. Ohio State University. Contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.


Mar. 26-28--MISSOURI ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Lodge of the Four Seasons, Lake of the Ozarks, MO. Contact: Erwin Gadd, Bureau of Community Sanitation, Missouri Division of Health, Box 570, Jefferson City, MO 65101.

Mar. 27-28--WESTERN FOOD INDUSTRY CONFERENCE. University of California, Davis, CA. Contact: John C. Bruhn, Extension Food Technologist, Dept. of Food Science & Technology, University of California, Davis, CA, 916-752-2192.

Mar. 28--ONTARIO MILK AND FOOD SANITARIANS ASSOCIATION. Annual Meeting, Holiday Inn, Airport Rd., Toronto. Contact: Roger Wray, 23 Windsor St., Guelph Ont., N1E 3N2, Canada.

April 10-11--PRESENT AND FUTURE TRENDS IN FOOD AND BEVERAGE PACKAGING, Short Course. Holiday Inn, Clemson, SC. Contact: T. C. Titus, Food Science Dept., Clemson University, Clemson, SC 29631.


June 5--PENNSYLVANIA SANITARIANS ASSOCIATION, Annual Meeting. Keller Conference Center, Pennsylvania State University, University Park Campus, State College, PA 16801. Contact: Sid Barnard, Pennsylvania State University.

June 10-13--INSTITUTE OF FOOD TECHNOLOGISTS 39TH ANNUAL MEETING AND FOOD EXPO. Alfonso J. Cervantes Convention and Exhibition Center, St. Louis, MO. Contact: C. L. Willey, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601.

con’t p. 87
AWARDS NOMINATION FORM

NOMINATIONS FOR 1979 AWARDS

Please Print

I wish to place the following person(s) in nomination for the IAMFES Award(s) as listed below. Biographical and supporting information on the candidate(s) will be supplied by me upon request.

SANITARIAN’S AWARD. $1000 to a County or Municipal sanitarian, who during the past seven years has made outstanding contributions to the health and welfare of his community.

Name ____________________________ Address ____________________________
City ____________________________ State ____________________________ Zip Code ____________

EDUCATOR-INDUSTRY AWARD. $1000 to an educator who during the past seven years has made outstanding contributions to food safety and sanitation.

Name ____________________________ Address ____________________________
City ____________________________ State ____________________________ Zip Code ____________

CITATION AWARD. To a member who has given outstanding service to IAMFES in fulfilling its objectives.

Name ____________________________ Address ____________________________
City ____________________________ State ____________________________ Zip Code ____________

HONORARY LIFE MEMBERSHIP. To a member who has given long and outstanding service to IAMFES.

Name ____________________________ Address ____________________________
City ____________________________ State ____________________________ Zip Code ____________

SUBMITTING IAMFES MEMBER:

Name ____________________________ Address ____________________________
City ____________________________ State ____________________________ Zip Code ____________
Telephone number ____________________________

Nominations must be returned by April 1, 1979 to: Henry V. Atherton, Chairman of IAMFES Recognition and Awards Committee, Dairy Building, University of Vermont, Burlington, Vermont 05401.
Nominees must be an active member.
For an enjoyable, educational, and a memorable professional meeting...

Plan now to attend the 66th Annual Meeting of IAMFES, August 12-16, 1979

at the Sheraton Twin Towers, Orlando, Florida

For years IAMFES Annual Meetings have offered informative group sessions and discussions in a relaxed and enjoyable atmosphere.

This year's meeting will have all that and more. It provides you the opportunity of sharing the visit with your family. Plan your vacation around the meeting. Orlando, one of the leading vacation resort areas in the United States, offers Disney World, Sea World, Circus World, Kennedy Space Center and many other places of interest for you and your family to enjoy.

As a departure from previous programs, a free afternoon will be available for you to visit the attractions of your choice.

Make plans now to meet in Orlando for an enriching professional program in an enjoyable setting.

1979 I.A.M.F.E.S. ANNUAL MEETING

Advance Registration Form for the 66th Annual Meeting, August 12-16, 1979, Orlando, Florida

MAIL TO: Jay Boosinger, Co-Chairman of Registration
IAMFES
Florida Dept. of Agriculture & Consumer Services
Division of Dairy Industry
508 Mayo Building
Tallahassee, Florida 32304

Please check where applicable:
Affiliate Delegate Speaker
Past President Host
Executive Board

Make checks payable to: IAMFES - 1979 Meeting Fund

Advance register and save - refundable if you don't attend

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Student (No Charge)

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Name __________________________________________ Name __________________________________________
Children's First Names and Age ____________________________________________________________
Affiliate or Company ____________________________________________________________
Address
City
State ___________________ Zip ___________________
Means of Transportation ____________________________________________________________

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Telephone: 305-351-1000 305-351-2311 1-800-327-2110

Arrival Date ____________
Arrival Time ____________
Name _____________________
Address _____________________
City _____________________ State _____________________ Zip _____________________
Means of Transportation ____________________________________________________________

Please check type of accommodation required
- Single (one person) $30.00
- Double (two persons) $38.00
- Triple - $43.00
- Quadruple - $48.00
- King Corner - $60.00
- Parlor Suite - $90.00
- Penthouse - $225.00

Limited roll-a-ways available at $5.00 each.

Family Plan: There is no charge for children when in same room with parents.

Send directly to: Reservations, Sheraton Twin Towers

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Reservations must be received by July 27, 1979
Reservations will be held until 6:00 P.M.
unless a later hour is specified (with deposit)
Check in after 3:00 P.M.
Check out time is 12:00 Noon

Parlor Suite - $90.00
Penthouse - $225.00
The 18th Annual Meeting of the National Mastitis Council will be held Tuesday, February 19 through Thursday, February 21, 1979. Program highlights will include presentations and technology sessions on the following subjects:

- **SOMATIC CELLS-NATURE & ACTION**
- **LEUKOCYTE MIGRATION DURING MASTITIS**
- **EXPERIENCES WITH SOMATIC CELL COUNTING ON DHI**
- **THE ROLE OF SOMATIC CELL COUNTING IN THE NATIONAL DHI PROGRAM**
- **SOMATIC CELLS IN GOAT MILK**
- **RELATIONSHIP OF MILK CHARACTERISTICS AND MILKING PROCEDURES TO MASTITIS**
- **PRESENT STATUS OF MASTITIS VACCINATION**
- **OVERVIEW OF CURRENT RECOMMENDATIONS FOR MASTITIS CONTROL AND HOW TO IMPLEMENT THEM**

**LABORATORY PROCEDURES IN SOMATIC CELL COUNTING**
- Fossomatic
- Filter-DNA
- Coulter Counter
- Technicon

**LABORATORY PROCEDURES IN ANTIBIOTIC RESIDUES**
- Bacillus Stearothermophilus
- Charm Test

**FARM PRACTICES**
- Checking Milking Equipment
- Milking Procedures and Cow Management
- Cleaning and Sanitation
- Selling Mastitis Management

**DHI SOMATIC CELL COUNTING PROGRAM**
- **MASTITIS THERAPY**
  - Pharmacokinetic Principles of Intramammary Therapy
  - Infection Rates and Response to Therapy in Ten Saskatchewan Dairies
  - Evaluation of a Method of Treating Clinical Infections
  - Field observations in Mastitis Therapy

**Discussant**

For additional information or to register, contact the National Mastitis Council, 30 F Street, N.W., Washington, D.C., 20001, 202-393-6607.
CLASSIFIED AD

For Sale

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

Index to Advertisers

Babson Bros. ............... Outside Back Cover
Haynes Manufacturing ........... Inside Back Cover
Klenzade ................. 1
National Mastitis Council ............. 93
National Sanitation Foundation .... Inside Front Cover
Norton Plastics ................. 94
Reitman Manufacturing ............. 96
Worldwide Finance Exchange ......... 95

APPLICATION FOR MEMBERSHIP

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Name __________________________ Date ____________
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________________________________________ Zip Code
Is the above your _________ business _________ home address?

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Affiliate membership—through an affiliate—$18.00 plus affiliate dues (contact your local affiliate secretary) (Eff. July 1, 1979)
Student Membership $5.00 with verification of student status. Student subscription effective from receipt of payment to the end of the calendar year.

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Recommended by the U. S. Public Health Service

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MOLDED TO PRECISION STANDARDS

LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE Advantages

Tight joints, no leaks, no shrinkage

Sanitary, unaffected by heat or fats

Non-porous, no seams or crevices

Odorless, polished surfaces, easily cleaned

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Time-saving, easy to assemble

Self-centering

No sticking to fittings

Eliminate line blocks

Help overcome line vibrations

Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.

Pack 100 to the box. Order through your dairy supply house.

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THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

SIZES 1" - 1½"

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Gasket Color:

- slightly off-white

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

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

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ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

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SPRAY - PACKED 6 - 16 OZ. CANS PER CARTON

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Stimulation: The First Step Toward A Better Harvest

Dr. Richard D. Mochrie
Professor, Animal Science
No. Carolina State University

It's milking time, and you are ready once more to harvest that milk crop you continue to work so hard for. You have provided the best nutrients to these dairy animals, and the best housing. You have raised them from calves and provided them with every benefit at your disposal to assure that they will be good producers. And now, they have just entered your milking parlor or are ready to be milked in the barn. Your next step, stimulation, will either make all your work worthwhile, or negate much of the effort you have put in.

Oxytocin Means Let Down
Stimulation is more than cleaning the udder before milking. Properly done, stimulation substitutes completely for the natural signal provided by the calf to tell the cow she is hungry. Oxytocin, a hormone released into the blood stream after stimulation, signals the milk making glands (alveoli) to release the milk they have produced. This squeezing out of tiny droplets of milk from each of the millions of alveoli is called "let-down." The let-down is directly related to the amount of oxytocin in the blood stream, and the amount of oxytocin present is directly related to the thoroughness of the stimulation.

Complementary Milk: Profits Left in the Udder
In tests conducted on a number of herds, we found that from three percent to twenty percent more milk was present in the udder than was being harvested, partly due to inadequate stimulation. The animals were first stimulated and milked in the normal way, by their regular milker, and production recorded just before complementary was obtained. Later, the cows were stimulated as usual and then, just before attaching the milking machine, they were injected with an adequate amount of oxytocin. The average cow gave in the area of ten percent more milk after receiving maximum stimulation with the additional oxytocin. This ten percent as complementary milk (instead of being part of the normal) represents profit lost for three reasons: First, this milk would not have been harvested during a normal milking. Second, the last of the milk is always richer in fat, and so the fat test would be lower. And last, with the complementary milk remaining in the alveoli, the cells become less active in producing milk. Over a normal lactation period, this can make a good cow produce far less than she is capable of. With proper stimulation, the amount of complementary can be reduced to about the same minimum as injecting oxytocin.

Thirty Seconds of Profitable Time
All results point to the fact that about thirty seconds is the amount of time necessary to achieve maximum stimulation and proper cleaning. This should be a vigorous massage—preferably with a disposable paper towel. Less time fails to provide the amount of needed oxytocin, and more than thirty seconds of stimulation does not increase the level. Time spent stimulating the animal will determine if she has received an adequate natural signal to allow maximum let-down. The thirty seconds you spend on each cow to assure proper stimulation may well be the most profitable time you use on the farm.