

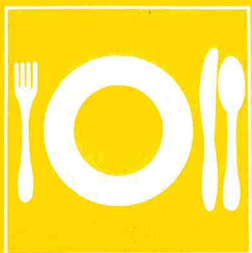
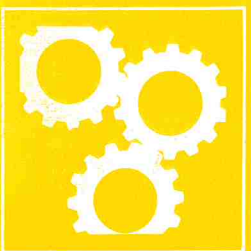
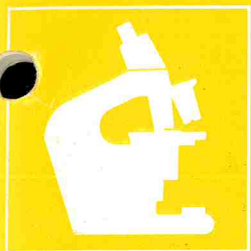
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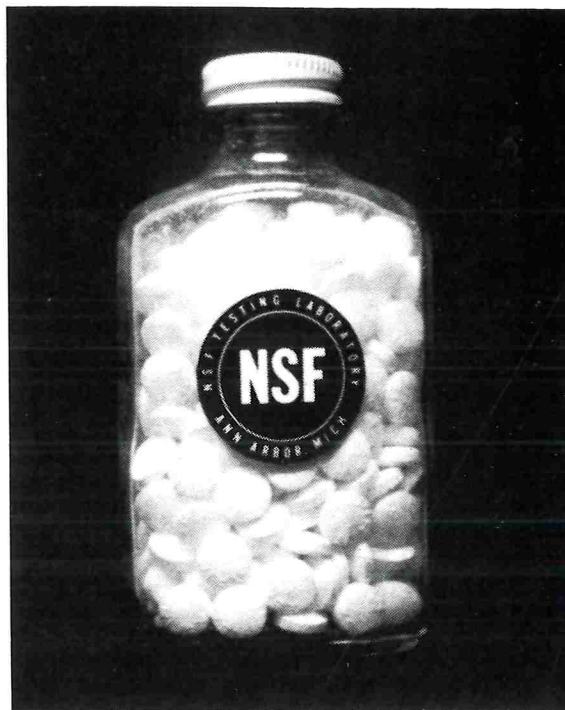
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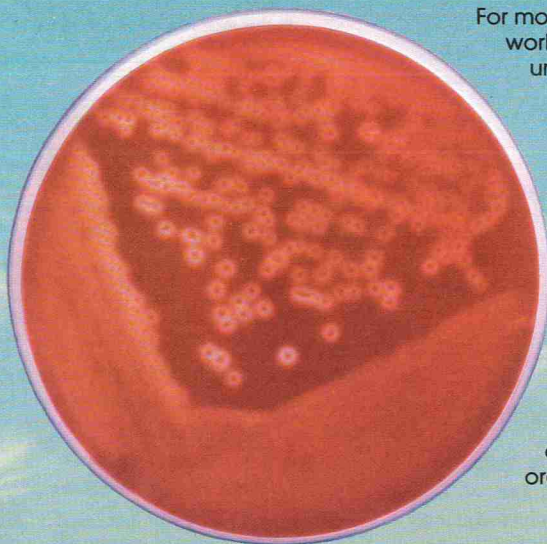
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## Appearance of Beef, Pork and Lamb Stored in Vacuum or Modified Gas Atmospheres

S. C. SEIDEMAN, C. VANDERZANT\*, G. C. SMITH, C. W. DILL and Z. L. CARPENTER

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(Received for publication July 9, 1979)

### ABSTRACT

Beef, pork and lamb loins were vacuum-packaged as subprimal cuts, vacuum-packaged as reformed subprimal cuts, vacuum-packaged as retail cuts or packaged in modified gas atmospheres as retail cuts. Cuts were stored and evaluated, after 0, 7, 14 or 21 days of storage, for surface discoloration and overall appearance. Gas analysis of the headspace of packages was also obtained. In all vacuum packaging treatments, the percentage of CO<sub>2</sub> increased and the percentage of O<sub>2</sub> decreased with increased storage. The gas composition in modified gas atmosphere packages changed little during the 21-day study. Using systems and gas combinations involved in the present study, retail cuts of beef, pork and lamb could not be successfully precut, retail-packaged and stored for 7 to 21 days in vacuum or modified gas atmospheres. Vacuum-packaged retail cuts and retail cuts stored in modified gas atmospheres sustained extensive surface discoloration and were of unsatisfactory appearance, even after only 7 days of storage and 1 day of retail display. Conversely, storage of retail cuts as vacuum-packaged reformed subprimal cuts for 7 to 21 days decreased ( $P < .05$ ) surface discoloration and improved ( $P < .05$ ) overall appearance in comparison to retail cuts from loins stored as subprimals for comparable times in vacuum packages.

Vacuum packaging currently is a preferred means of packaging beef and lamb because it tends to minimize shrink loss, discoloration and microbial growth as compared to the shipment of either unprotected wholesale cuts or carcasses. However, several disadvantages persist: (a) vacuum-packaged bone-in primal cuts can have leaker rates as high as 40%, and (b) amount of purge may be increased and cuts are often distorted due to vacuum packaging (15). Modified gas atmosphere packaging could reduce leaker rates, purge loss and distortion.

Centralized production of retail meat cuts continues to be intensely researched by the meat industry. Due to recent increases in costs for transportation, labor, equipment and storage space, many retailers are critically evaluating the cost of conventional retail meat operations. Processing of carcasses into trimmed subprimal cuts at a central location and subsequent distribution to retail stores offers an alternative solution to economic problems associated with breaking-cutting-trimming at retail store level.

The so-called "boxed beef concept" derives its name from the fact that trimmed subprimal cuts are very often vacuum-packaged and shipped in corrugated cardboard boxes to retail stores. Very recently, there have been

attempts to further process subprimal cuts at a central location by cutting and trimming to form actual retail cuts and then "reforming" the subprimal cuts followed by vacuum packaging for transport-distribution to retail stores. Other meat retailers are attempting to perform complete retail cut preparation, including wrapping, at a central location for subsequent distribution to retail outlets.

In recent years, there has been a renewed interest in modified gas atmosphere packaging. Since some gases produce beneficial results relative to meat color while others only inhibit bacterial activity, mixtures of two or more gases are being considered for possible use in the storage of meats for greater shelf life. Most of the previous research indicates that a concentration of 20-30% carbon dioxide is sufficient to prevent growth of aerobic spoilage bacteria (3,15). Ledward (9) reported that high concentrations of carbon dioxide (over 20%) will discolor meat; however, Taylor (15) determined that 50-80% CO<sub>2</sub> is often found in residual air spaces in vacuum packages with no detrimental effect on meat color. Correspondingly, the objective of the present study was to compare the surface discoloration and appearance of beef, pork and lamb retail cuts, derived from vacuum-packaged subprimal or vacuum-packaged reformed subprimal cuts, or which had been stored, as retail cuts, in vacuum or modified gas atmospheres.

### MATERIALS AND METHODS

#### *Packaging*

Forty-two animals (14 beef, 14 pork and 14 lamb) were processed according to normal slaughtering procedures in the Texas A&M University Meats Laboratory. Twenty-eight loins of each meat type were removed from carcasses 3-5 days postmortem. Each of these loins was subdivided into 2-3 subprimal cuts. Beef, pork and lamb were packaged as subprimal cuts, reformed subprimal cuts or as retail cuts (steaks or chops) using a CVP vacuum packaging machine operated at the maximum vacuumizing capacity of the machine (746.7 mm of Hg). Reformed subprimals were prepared by cutting loins into retail cuts (2.5 cm in thickness), reforming the original subprimal cut by holding the steaks or chops together, placing the reformed cut in a bag, and vacuum packaging. Other loins were cut to yield retail cuts (2.5 cm in thickness). The retail cuts were placed on styrofoam trays and overwrapped with oxygen-permeable, polyvinyl chloride film. Groups of three retail cuts, after retail packaging, were placed in 3-shelf cardboard containers (Fig. 1). Each cardboard container was placed in a semi-permeable bag and vacuum-packaged. Retail cuts in cardboard containers were assigned, at random, to each of three treatments: (a)

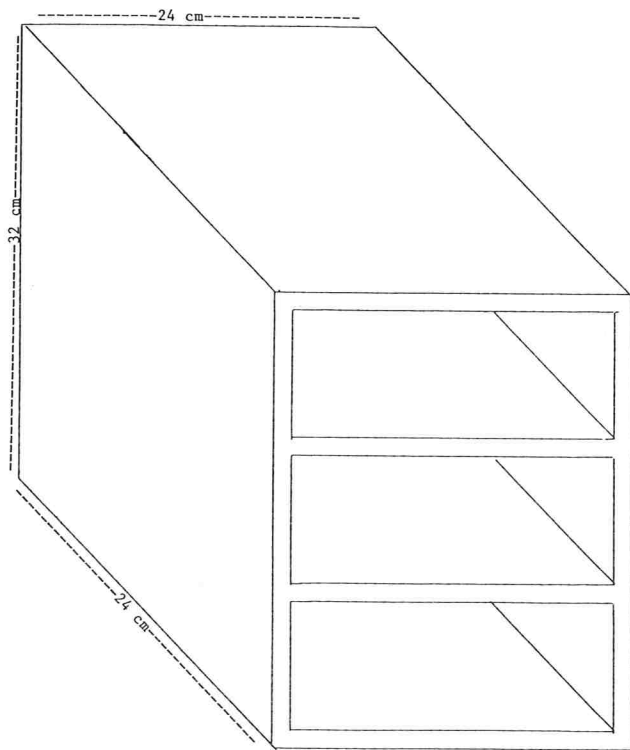


Figure 1. Cardboard container used for the storage of precut, prepackaged retail cuts.

one group remained under vacuum, (b) one group was injected with 1,700 cm<sup>3</sup> of a 20% CO<sub>2</sub> + 80% N<sub>2</sub> gas mixture and (c) one group was injected with 1,700 cm<sup>3</sup> of a 40% CO<sub>2</sub> + 60% N<sub>2</sub> gas mixture. A volume of 1,700 cm<sup>3</sup> of gas mixture was selected because this relieved distortion of the cardboard container caused by vacuum treatment. All subprimal, reformed subprimal and retail cuts were packaged in the same semi-permeable (polyethylene-nylon-surlyn laminate) film [Oxygen Transmission Rate (OTR) = 69 cc/m<sup>2</sup>/24 h; Carbon Dioxide Transmission Rate (CO<sub>2</sub>TR) = 206 cc/m<sup>2</sup>/24 h]. Packages were stored at 2 ± 1 C for periods of 0, 7, 14 and 21 days (Table 1). Certain samples were analyzed immediately after packaging (on day 0 of storage) for gas composition and appearance. Values obtained constituted initial data for comparative purposes.

#### Gas analysis

The relative weight percentages of oxygen, nitrogen and carbon

TABLE 1. Experimental design for the storage of beef, pork and lamb cuts.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                         |                 |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-----------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts     |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | 3                                | 3                       | 3               | 3                                      | 3                                      |
| 7                       | 3                                | 3                       | 3               | 3                                      | 3                                      |
| 14                      | 3                                | 3                       | 3               | 3                                      | 3                                      |
| 21                      | 3                                | 3                       | 3               | 3                                      | 3                                      |

<sup>a</sup>Number of cuts in each packaging treatment.

TABLE 2. Experimental design for retail display and evaluation of beef, pork and lamb cuts.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                         |                 |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-----------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts     |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | 9                                | 9                       | 9               | 9                                      | 9                                      |
| 7                       | 9                                | 9                       | 9               | 9                                      | 9                                      |
| 14                      | 9                                | 9                       | 9               | 9                                      | 9                                      |
| 21                      | 9                                | 9                       | 9               | 9                                      | 9                                      |

<sup>a</sup>Number of cuts in each packaging treatment.

dioxide were determined in the headspace of packaged meats. These analyses were repeated at weekly intervals during the 21-day storage period. Silicone glue was applied to a spot on the exterior of the packaging film and allowed to dry to facilitate sampling of the gas atmospheres. This provided a sampling area that would not tear when punctured and would reseal itself after sampling. Each package was sampled in duplicate for headspace gas analysis before the package was opened for other analyses. A 5-ml sample of headspace gas was drawn through an 18-gauge needle with a gas-tight syringe and immediately analyzed by gas chromatography. Fifty ml of helium were injected from a gas-tight syringe into vacuum packages at the termination of each storage period to create sufficient gas volume for sampling. Packages were equilibrated at 2 ± 1 C for 10 min before sampling the headspace gases.

The permanent gases were resolved in a Gow-Mac series 550 gas chromatograph fitted with two columns (1.52 m × 6.35 mm) in series (13) and operated at a helium flow rate of 50 ml/min. The first column was packed with 80-100 mesh porapak Q and the second column with 80-100 mesh Molecular Sieve 5A (11). The column oven was operated at 60 C and both the injector and detector were set at 100 C. The thermal conductivity detector was operated at a bridge current of 130 ma. Gas chromatographic results were quantitated with a Hewlett-Packard Model 3380A Integrator, which was calibrated with a standard gas mixture (Supelco) consisting of 50.43% CO<sub>2</sub>, 30.28% O<sub>2</sub>, 18.27% N<sub>2</sub> and 1.02% CO.

#### Retail evaluation

At the termination of each storage period, steaks or chops (2.5 cm in thickness) were cut from subprimal cuts, placed on styrofoam trays and overwrapped with oxygen-permeable, polyvinyl chloride film. Steaks or chops from reformed subprimals were placed on styrofoam trays and overwrapped with oxygen-permeable, polyvinyl chloride film. Steaks or chops stored as retail cuts were removed from storage containers and immediately displayed without rewrapping. The experimental design for this phase of the study is shown in Table 2.

Retail cuts were placed under simulated retail display conditions (1-3 C, 970 lux of incandescent light). A 3-member trained panel evaluated cuts initially and after 1, 3 and 5 days of retail display for surface discoloration by visual evaluation, employing a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration) and for overall appearance, using an 8-point scale (8 = extremely desirable; 1 = extremely undesirable). Cuts with coded identity were evaluated in completely random sequence; evaluators had no knowledge regarding treatment of a sample when they evaluated it.

#### Microbiological

Data regarding the microbial flora of cuts from this study are included in the companion report of Christopher et al. (2).

### Statistical analyses

Analysis of variance comparing data for vacuum-packaged subprimal cuts or data for retail cuts from subprimals stored in vacuum packages with data from every other treatment was performed within each of the three storage intervals. Comparisons were also made of data within each packaging treatment over the three storage periods. When significant ( $P < .05$ ) main effects were observed in the analysis of variance, mean separation was accomplished by use of the Student-Newman-Keuls test (6).

## RESULTS

### Beef

Relative weight percentages of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in the headspace of packaged beef at weekly storage intervals are included in Table 3. The initial percentages of O<sub>2</sub> and N<sub>2</sub> in vacuum packages were only slightly different from that of air. It is not clear whether very small air pockets were present or whether these gases volatilized from the meat into the space created by the injection of helium into the vacuum packages. The initial level of

CO<sub>2</sub> in the reformed subprimal cuts and vacuum-packaged retail cuts, was higher than that present in vacuum-packaged subprimal cuts, suggesting release of CO<sub>2</sub> from the fresh cut surfaces of these cuts. The O<sub>2</sub> concentration within the package of vacuum-packaged subprimal cuts decreased to a low level within 14 days. There was a concomitant increase in the CO<sub>2</sub> concentration, very likely due to respiration of surface bacteria or to evolution of CO<sub>2</sub> from meat tissues. The conversion of O<sub>2</sub> to CO<sub>2</sub> was much less noticeable in all other packages, all of which had higher initial CO<sub>2</sub> levels. The atmospheres in packages injected with modified atmospheres changed little during storage.

Mean surface discoloration ratings for beef steaks during retail display are presented in Table 4. Steaks stored for 14 or 21 days as retail cuts had more ( $P < .05$ ) surface discoloration than did steaks from vacuum packaged subprimal cuts after 1 (6 of 6 comparisons), 3 (6 of 6 comparisons) and 5 (2 of 6 comparisons) days of

TABLE 3. Relative weight percentages of carbon dioxide, oxygen and nitrogen in the package headspace of beef during the 3-week storage period.

| Storage interval (days) | Gas component   | Packaging treatment |                         |                   |                                        |                                        |
|-------------------------|-----------------|---------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|
|                         |                 | Vacuum packaged     |                         | Retail cuts       |                                        |                                        |
|                         |                 | Subprimal cuts      | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | CO <sub>2</sub> | 0.1 <sup>d</sup>    | 5.2 <sup>c</sup>        | 4.4 <sup>d</sup>  | 12.0 <sup>b</sup>                      | 21.9 <sup>b</sup>                      |
|                         | O <sub>2</sub>  | 21.9 <sup>a</sup>   | 17.7 <sup>a</sup>       | 20.5 <sup>a</sup> | 9.5 <sup>a</sup>                       | 9.2 <sup>a</sup>                       |
|                         | N <sub>2</sub>  | 78.0 <sup>b</sup>   | 76.7 <sup>a</sup>       | 75.0 <sup>b</sup> | 78.5 <sup>a</sup>                      | 68.9 <sup>b</sup>                      |
| 7                       | CO <sub>2</sub> | 10.5 <sup>c</sup>   | 11.4 <sup>b</sup>       | 8.3 <sup>c</sup>  | 11.3 <sup>b</sup>                      | 26.0 <sup>a</sup>                      |
|                         | O <sub>2</sub>  | 11.6 <sup>b</sup>   | 11.3 <sup>c</sup>       | 16.9 <sup>b</sup> | 9.8 <sup>a</sup>                       | 6.2 <sup>b</sup>                       |
|                         | N <sub>2</sub>  | 77.9 <sup>b</sup>   | 77.4 <sup>a</sup>       | 74.8 <sup>b</sup> | 78.9 <sup>a</sup>                      | 67.7 <sup>b</sup>                      |
| 14                      | CO <sub>2</sub> | 19.5 <sup>a</sup>   | 23.4 <sup>a</sup>       | 10.6 <sup>b</sup> | 14.1 <sup>a</sup>                      | 21.6 <sup>b</sup>                      |
|                         | O <sub>2</sub>  | 1.8 <sup>c</sup>    | 5.3 <sup>d</sup>        | 11.1 <sup>c</sup> | 6.8 <sup>a</sup>                       | 8.1 <sup>a</sup>                       |
|                         | N <sub>2</sub>  | 78.6 <sup>b</sup>   | 71.4 <sup>a</sup>       | 78.4 <sup>a</sup> | 79.1 <sup>a</sup>                      | 70.3 <sup>a</sup>                      |
| 21                      | CO <sub>2</sub> | 14.8 <sup>b</sup>   | 6.6 <sup>c</sup>        | 12.7 <sup>a</sup> | 10.7 <sup>b</sup>                      | 26.4 <sup>a</sup>                      |
|                         | O <sub>2</sub>  | 1.6 <sup>c</sup>    | 15.7 <sup>b</sup>       | 6.5 <sup>d</sup>  | 9.6 <sup>a</sup>                       | 4.7 <sup>b</sup>                       |
|                         | N <sub>2</sub>  | 83.6 <sup>a</sup>   | 77.6 <sup>a</sup>       | 80.9 <sup>a</sup> | 79.6 <sup>a</sup>                      | 68.9 <sup>a</sup>                      |

a,b,c,d Means of the same gas component in the same column bearing a common superscript letter do not differ ( $P > .05$ ).

TABLE 4. Mean values for surface discoloration<sup>a</sup> of beef steaks during retail display.

| Day of retail display | Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                                        |                                        |
|-----------------------|-------------------------|----------------------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|
|                       |                         | Vacuum packaged                  |                         | Retail cuts       |                                        |                                        |
|                       |                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| 1                     | Initial                 | 6.6 <sup>c</sup>                 | 6.6 <sup>c</sup>        | 6.6 <sup>c</sup>  | 6.6 <sup>c</sup>                       | 6.6 <sup>c</sup>                       |
|                       | 7                       | 5.0 <sup>d</sup>                 | 6.0 <sup>d</sup>        | 5.1 <sup>d</sup>  | 5.4 <sup>d</sup>                       | 3.4 <sup>e</sup>                       |
|                       | 14                      | 6.4 <sup>c</sup>                 | 6.6 <sup>c</sup>        | 5.2 <sup>d</sup>  | 3.0 <sup>e</sup>                       | 4.5 <sup>d</sup>                       |
|                       | 21                      | 6.7 <sup>c</sup>                 | 6.7 <sup>c</sup>        | 5.3 <sup>d</sup>  | 1.9 <sup>f</sup>                       | 2.9 <sup>e</sup>                       |
| 3                     | Initial                 | 5.1 <sup>c</sup>                 | 5.1 <sup>c</sup>        | 5.1 <sup>c</sup>  | 5.1 <sup>c</sup>                       | 5.1 <sup>c</sup>                       |
|                       | 7                       | 5.3 <sup>c</sup>                 | 5.0 <sup>c</sup>        | 4.5 <sup>c</sup>  | 5.2 <sup>c</sup>                       | 3.4 <sup>de</sup>                      |
|                       | 14                      | 5.5 <sup>c</sup>                 | 5.2 <sup>c</sup>        | 4.6 <sup>c</sup>  | 3.6 <sup>d</sup>                       | 4.4 <sup>cd</sup>                      |
|                       | 21                      | 5.5 <sup>c</sup>                 | 5.1 <sup>c</sup>        | 4.3 <sup>c</sup>  | 1.3 <sup>e</sup>                       | 2.5 <sup>e</sup>                       |
| 5                     | Initial                 | 5.1 <sup>c</sup>                 | 5.1 <sup>c</sup>        | 5.1 <sup>c</sup>  | 5.1 <sup>c</sup>                       | 5.1 <sup>c</sup>                       |
|                       | 7                       | 4.9 <sup>cd</sup>                | 4.9 <sup>c</sup>        | 4.7 <sup>cd</sup> | 5.5 <sup>c</sup>                       | 3.5 <sup>d</sup>                       |
|                       | 14                      | 4.0 <sup>d</sup>                 | 3.6 <sup>d</sup>        | 3.3 <sup>e</sup>  | 3.1 <sup>d</sup>                       | 3.8 <sup>d</sup>                       |
|                       | 21                      | 4.7 <sup>cd</sup>                | 3.4 <sup>d</sup>        | 3.8 <sup>de</sup> | 1.5 <sup>e</sup>                       | 3.3 <sup>d</sup>                       |

<sup>a</sup>Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different ( $P < .05$ ) from means obtained for cuts derived from vacuum packaged subprimal cuts.

c,d,e,f Means in the same column and same day of display bearing a common superscript letter do not differ ( $P > .05$ ).



retail display. The amount of surface discoloration on steaks stored as retail cuts in modified gas atmospheres steadily increased and they are almost totally discolored after 14 days of storage and 1, 3 or 5 days of retail display.

Mean overall appearance ratings for beef steaks are presented in Table 5. Steaks stored as retail cuts for 14 and 21 days had less desirable overall appearance than steaks from vacuum-packaged subprimal cuts after 1 (6 of 6 comparisons), 3 (6 of 6 comparisons) and 5 (5 of 6 comparisons) days of retail display. Between initial packaging and 7 days of storage, overall appearance ratings decreased to the greatest extent for retail cuts stored in an atmosphere originally containing 40% CO<sub>2</sub>-60% N<sub>2</sub>. A similar decrease in overall appearance ratings occurred between the 7th and 14th days of storage for those steaks originally stored in an atmosphere containing 20% CO<sub>2</sub>-80% N<sub>2</sub>.

*Pork*

Relative weight percentages of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in the headspace samples withdrawn from the packages of pork are presented in Table 6. The weight percentage of CO<sub>2</sub> from packages of subprimal cuts, reformed subprimal cuts and retail cuts which were vacuum-packaged significantly increased during storage while the percentage of O<sub>2</sub> decreased. The weight percentages of O<sub>2</sub> and N<sub>2</sub> were relatively constant after 1 week of storage for those packages initially injected with modified gas atmospheres. In these packages the CO<sub>2</sub> concentration decreased during the first week of storage and then remained fairly constant for the remaining 2 weeks of storage.

Mean surface discoloration ratings for retail pork chops are shown in Table 7. Chops stored as reformed subprimals had significantly less surface discoloration than did chops obtained from vacuum-packaged

TABLE 5. Mean values for overall appearance<sup>a</sup> ratings of beef steaks during retail display.

| Day of retail display | Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                                        |                                        |                   |
|-----------------------|-------------------------|----------------------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|-------------------|
|                       |                         | Vacuum packaged                  |                         | Retail cuts       |                                        |                                        |                   |
|                       |                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                   |
| 1                     | Initial                 | 7.3 <sup>c</sup>                 | 7.3 <sup>c</sup>        | 7.3 <sup>c</sup>  | 7.3 <sup>c</sup>                       | 7.3 <sup>c</sup>                       | 7.3 <sup>c</sup>  |
|                       | 7                       | 5.7 <sup>d</sup>                 | 6.5 <sup>d</sup>        | 5.0 <sup>d</sup>  | 5.6 <sup>d</sup>                       | 2.8 <sup>e</sup>                       | 2.8 <sup>e</sup>  |
|                       | 14                      | 6.8 <sup>c</sup>                 | 7.0 <sup>c</sup>        | 4.9 <sup>d</sup>  | 2.8 <sup>e</sup>                       | 4.4 <sup>d</sup>                       | 4.4 <sup>d</sup>  |
|                       | 21                      | 7.4 <sup>c</sup>                 | 7.2 <sup>c</sup>        | 4.6 <sup>d</sup>  | 1.8 <sup>f</sup>                       | 2.3 <sup>e</sup>                       | 2.3 <sup>e</sup>  |
| 3                     | Initial                 | 5.5 <sup>d</sup>                 | 5.5 <sup>c</sup>        | 5.5 <sup>c</sup>  | 5.5 <sup>c</sup>                       | 5.5 <sup>c</sup>                       | 5.5 <sup>c</sup>  |
|                       | 7                       | 5.8 <sup>cd</sup>                | 5.8 <sup>c</sup>        | 4.9 <sup>cd</sup> | 5.6 <sup>c</sup>                       | 3.2 <sup>de</sup>                      | 3.2 <sup>de</sup> |
|                       | 14                      | 5.7 <sup>cd</sup>                | 6.0 <sup>c</sup>        | 4.2 <sup>d</sup>  | 3.6 <sup>d</sup>                       | 4.0 <sup>d</sup>                       | 4.0 <sup>d</sup>  |
|                       | 21                      | 6.5 <sup>c</sup>                 | 6.0 <sup>c</sup>        | 3.9 <sup>d</sup>  | 1.3 <sup>e</sup>                       | 2.3 <sup>e</sup>                       | 2.3 <sup>e</sup>  |
| 5                     | Initial                 | 5.7 <sup>c</sup>                 | 5.7 <sup>c</sup>        | 5.7 <sup>c</sup>  | 5.7 <sup>c</sup>                       | 5.7 <sup>c</sup>                       | 5.7 <sup>c</sup>  |
|                       | 7                       | 5.5 <sup>c</sup>                 | 5.4 <sup>c</sup>        | 5.0 <sup>c</sup>  | 5.3 <sup>c</sup>                       | 3.2 <sup>d</sup>                       | 3.2 <sup>d</sup>  |
|                       | 14                      | 4.0 <sup>d</sup>                 | 3.8 <sup>d</sup>        | 2.8 <sup>d</sup>  | 2.6 <sup>d</sup>                       | 3.7 <sup>d</sup>                       | 3.7 <sup>d</sup>  |
|                       | 21                      | 4.5 <sup>d</sup>                 | 2.9 <sup>e</sup>        | 3.3 <sup>d</sup>  | 1.7 <sup>e</sup>                       | 3.0 <sup>d</sup>                       | 3.0 <sup>d</sup>  |

<sup>a</sup>Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different (P < .05) from means obtained for cuts derived from vacuum packaged subprimal cuts.

<sup>c,d,e,f</sup>Means in the same column and for the same day of display bearing a common superscript letter do not differ (P > .05).

TABLE 6. Relative weight percentages of carbon dioxide, oxygen and nitrogen in package headspace of pork during the 3-week storage interval.

| Storage interval (days) | Gas component   | Packaging treatment |                         |                   |                                        |                                        |
|-------------------------|-----------------|---------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|
|                         |                 | Vacuum packaged     |                         | Retail cuts       |                                        |                                        |
|                         |                 | Subprimal cuts      | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | CO <sub>2</sub> | 6.1 <sup>b</sup>    | 3.9 <sup>b</sup>        | 1.6 <sup>c</sup>  | 17.2 <sup>a</sup>                      | 28.6 <sup>a</sup>                      |
|                         | O <sub>2</sub>  | 19.5 <sup>a</sup>   | 19.9 <sup>a</sup>       | 21.3 <sup>a</sup> | 3.3 <sup>b</sup>                       | 5.8 <sup>a</sup>                       |
|                         | N <sub>2</sub>  | 74.5 <sup>b</sup>   | 76.5 <sup>c</sup>       | 77.0 <sup>b</sup> | 79.4 <sup>a</sup>                      | 65.6 <sup>b</sup>                      |
| 7                       | CO <sub>2</sub> | 6.8 <sup>b</sup>    | 9.8 <sup>a</sup>        | 6.1 <sup>b</sup>  | 13.2 <sup>b</sup>                      | 23.6 <sup>b</sup>                      |
|                         | O <sub>2</sub>  | 17.9 <sup>a</sup>   | 11.6 <sup>b</sup>       | 18.3 <sup>b</sup> | 7.7 <sup>a</sup>                       | 8.5 <sup>a</sup>                       |
|                         | N <sub>2</sub>  | 75.5 <sup>bc</sup>  | 78.6 <sup>b</sup>       | 75.7 <sup>c</sup> | 79.1 <sup>a</sup>                      | 68.0 <sup>a</sup>                      |
| 14                      | CO <sub>2</sub> | 19.5 <sup>a</sup>   | 11.2 <sup>a</sup>       | 7.3 <sup>b</sup>  | 12.0 <sup>b</sup>                      | 24.6 <sup>b</sup>                      |
|                         | O <sub>2</sub>  | 9.7 <sup>b</sup>    | 13.9 <sup>b</sup>       | 16.4 <sup>c</sup> | 9.8 <sup>a</sup>                       | 6.5 <sup>a</sup>                       |
|                         | N <sub>2</sub>  | 70.9 <sup>b</sup>   | 74.8 <sup>c</sup>       | 76.2 <sup>c</sup> | 78.2 <sup>a</sup>                      | 68.8 <sup>a</sup>                      |
| 21                      | CO <sub>2</sub> | 14.0 <sup>a</sup>   | 13.7 <sup>a</sup>       | 13.9 <sup>a</sup> | 13.1 <sup>b</sup>                      | 22.8 <sup>b</sup>                      |
|                         | O <sub>2</sub>  | 6.2 <sup>c</sup>    | 3.6 <sup>c</sup>        | 6.4 <sup>d</sup>  | 8.0 <sup>a</sup>                       | 7.0 <sup>a</sup>                       |
|                         | N <sub>2</sub>  | 79.8 <sup>a</sup>   | 82.7 <sup>a</sup>       | 79.8 <sup>a</sup> | 78.9 <sup>a</sup>                      | 70.3 <sup>a</sup>                      |

<sup>a,b,c,d</sup>Means of the same gas component in the same column bearing a common superscript letter do not differ (P > .05).

subprimal cuts after 21 days of storage and 1 or 3 days of retail display. These differences warrant further study of reformed subprimals as a possible method for distribution of pork chops. Chops stored as retail cuts had significantly more surface discoloration than chops from vacuum-packaged subprimal cuts after 7 (8 of 9 comparisons), 14 (9 of 9 comparisons) or 21 (8 of 9 comparisons) days of storage, and 1, 3 or 5 days of retail display.

Mean overall appearance ratings for retail pork chops are presented in Table 8. Chops stored as reformed subprimals had significantly higher overall appearance ratings than did chops from vacuum-packaged subprimal cuts after 1 (3 of 3 comparisons) and 3 (2 of 3 comparisons) days of retail display. Chops stored as retail

cuts had significantly lower overall appearance ratings than chops from vacuum-packaged subprimal cuts after 7 (5 of 9 comparisons), 14 (4 of 9 comparisons) or 21 (8 of 9 comparisons) days of storage, and 1, 3 or 5 days of retail display.

#### Lamb

The relative weight percentages of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in the headspace of packages of lamb cuts are shown in Table 9. Gas analysis of samples withdrawn from packages containing subprimal cuts and reformed subprimal cuts which were vacuum-packaged, showed a slow but consistent increase in the relative weight percentage of CO<sub>2</sub> and a decrease in the relative weight percentage of O<sub>2</sub> during the 21-day storage period. Gas

TABLE 7. Mean values for surface discoloration<sup>a</sup> of pork chops during retail display.

| Day of retail display | Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                  |                                        |
|-----------------------|-------------------------|----------------------------------|-------------------------|-------------------|------------------|----------------------------------------|
|                       |                         | Vacuum packaged                  |                         | Vacuum packaged   | Retail cuts      |                                        |
|                       |                         | Subprimal cuts                   | Reformed subprimal cuts |                   |                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> |
| 1                     | Initial                 | 5.3 <sup>c</sup>                 | 5.3 <sup>d</sup>        | 5.3 <sup>c</sup>  | 5.3 <sup>c</sup> | 5.3 <sup>c</sup>                       |
|                       | 7                       | 5.3 <sup>c</sup>                 | 5.8 <sup>cd</sup>       | 3.5 <sup>d</sup>  | 4.2 <sup>d</sup> | 2.6 <sup>d</sup>                       |
|                       | 14                      | 5.0 <sup>c</sup>                 | 6.1 <sup>cd</sup>       | 2.8 <sup>e</sup>  | 2.7 <sup>e</sup> | 2.6 <sup>d</sup>                       |
|                       | 21                      | 5.0 <sup>c</sup>                 | 6.6 <sup>c</sup>        | 2.0 <sup>f</sup>  | 1.7 <sup>f</sup> | 1.3 <sup>d</sup>                       |
| 3                     | Initial                 | 4.0 <sup>d</sup>                 | 4.0 <sup>d</sup>        | 4.0 <sup>c</sup>  | 4.0 <sup>c</sup> | 4.0 <sup>c</sup>                       |
|                       | 7                       | 5.2 <sup>c</sup>                 | 5.8 <sup>c</sup>        | 3.9 <sup>c</sup>  | 4.2 <sup>c</sup> | 2.7 <sup>c</sup>                       |
|                       | 14                      | 4.3 <sup>d</sup>                 | 5.3 <sup>c</sup>        | 2.4 <sup>d</sup>  | 2.8 <sup>d</sup> | 2.4 <sup>d</sup>                       |
|                       | 21                      | 3.8 <sup>d</sup>                 | 5.6 <sup>c</sup>        | 2.2 <sup>d</sup>  | 1.6 <sup>e</sup> | 1.8 <sup>d</sup>                       |
| 5                     | Initial                 | 3.9 <sup>c</sup>                 | 3.9 <sup>d</sup>        | 3.9 <sup>c</sup>  | 3.9 <sup>c</sup> | 3.9 <sup>c</sup>                       |
|                       | 7                       | 4.4 <sup>c</sup>                 | 5.1 <sup>c</sup>        | 3.6 <sup>cd</sup> | 3.7 <sup>c</sup> | 2.7 <sup>d</sup>                       |
|                       | 14                      | 2.6 <sup>d</sup>                 | 3.7 <sup>d</sup>        | 2.2 <sup>e</sup>  | 1.9 <sup>d</sup> | 2.0 <sup>d</sup>                       |
|                       | 21                      | 3.6 <sup>cd</sup>                | 3.7 <sup>d</sup>        | 2.8 <sup>de</sup> | 2.0 <sup>d</sup> | 2.1 <sup>d</sup>                       |

<sup>a</sup>Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different ( $P < .05$ ) from means obtained for cuts from vacuum packaged subprimal cuts.

<sup>c,d,e,f</sup>Means in the same column and for the same day of display bearing a common superscript letter do not differ ( $P > .05$ ).

TABLE 8. Mean values for overall appearance<sup>a</sup> ratings of pork chops during retail display.

| Day of retail display | Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                  |                                        |
|-----------------------|-------------------------|----------------------------------|-------------------------|-------------------|------------------|----------------------------------------|
|                       |                         | Vacuum packaged                  |                         | Vacuum packaged   | Retail cuts      |                                        |
|                       |                         | Subprimal cuts                   | Reformed subprimal cuts |                   |                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> |
| 1                     | Initial                 | 5.6 <sup>c</sup>                 | 5.6 <sup>d</sup>        | 5.6 <sup>c</sup>  | 5.6 <sup>c</sup> | 5.6 <sup>c</sup>                       |
|                       | 7                       | 5.6 <sup>c</sup>                 | 6.5 <sup>cd</sup>       | 3.2 <sup>d</sup>  | 4.8 <sup>d</sup> | 2.6 <sup>d</sup>                       |
|                       | 14                      | 5.1 <sup>cd</sup>                | 6.6 <sup>cd</sup>       | 3.1 <sup>d</sup>  | 2.8 <sup>e</sup> | 2.4 <sup>d</sup>                       |
|                       | 21                      | 4.7 <sup>d</sup>                 | 7.1 <sup>c</sup>        | 2.1 <sup>d</sup>  | 2.0 <sup>e</sup> | 2.0 <sup>d</sup>                       |
| 3                     | Initial                 | 4.4 <sup>cd</sup>                | 4.4 <sup>d</sup>        | 4.4 <sup>c</sup>  | 4.4 <sup>c</sup> | 4.4 <sup>c</sup>                       |
|                       | 7                       | 4.9 <sup>c</sup>                 | 6.2 <sup>c</sup>        | 3.5 <sup>cd</sup> | 4.5 <sup>c</sup> | 2.4 <sup>d</sup>                       |
|                       | 14                      | 4.1 <sup>d</sup>                 | 5.3 <sup>cd</sup>       | 2.5 <sup>d</sup>  | 2.1 <sup>d</sup> | 2.6 <sup>d</sup>                       |
|                       | 21                      | 3.5 <sup>d</sup>                 | 6.2 <sup>c</sup>        | 2.2 <sup>d</sup>  | 2.0 <sup>d</sup> | 2.0 <sup>d</sup>                       |
| 5                     | Initial                 | 3.8 <sup>d</sup>                 | 3.8 <sup>d</sup>        | 3.8 <sup>c</sup>  | 3.8 <sup>c</sup> | 3.8 <sup>c</sup>                       |
|                       | 7                       | 4.8 <sup>c</sup>                 | 5.6 <sup>c</sup>        | 3.6 <sup>c</sup>  | 3.8 <sup>c</sup> | 2.3 <sup>d</sup>                       |
|                       | 14                      | 2.7 <sup>e</sup>                 | 3.5 <sup>d</sup>        | 2.2 <sup>d</sup>  | 2.1 <sup>d</sup> | 2.7 <sup>d</sup>                       |
|                       | 21                      | 2.9 <sup>e</sup>                 | 3.5 <sup>d</sup>        | 2.3 <sup>d</sup>  | 1.9 <sup>d</sup> | 1.9 <sup>d</sup>                       |

<sup>a</sup>Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different ( $P < .05$ ) from means obtained for cuts from vacuum packaged subprimal cuts.

<sup>c,d,e</sup>Means in the same column and for the same day of display bearing a common superscript letter do not differ ( $P > .05$ ).

samples withdrawn from packages containing retail lamb chops stored in modified gas atmospheres showed a slight decrease in the relative weight percentage of CO<sub>2</sub> and an increase in the relative weight percentage of O<sub>2</sub> during the 21-day storage period.

Mean surface discoloration ratings for retail lamb chops are presented in Table 10. Lamb chops from reformed subprimals stored for 7 days had significantly less surface discoloration at days 1, 3 and 5 of retail display than did chops from vacuum-packaged subprimal cuts. Chops stored as retail cuts had significantly more surface discoloration than chops from vacuum-packaged subprimal cuts after 7 (7 of 9 comparisons), 14 (9 of 9 comparisons) or 21 (7 of 9 comparisons) days of storage, and 1, 3 or 5 days of retail display.

Mean overall appearance ratings for retail lamb chops are shown in Table 11. Chops stored as reformed subprimals for 7 days received significantly higher appearance ratings than chops from vacuum-packaged

subprimal cuts after 1, 3 or 5 days of retail display. Chops stored as retail cuts had significantly lower overall appearance ratings than chops from vacuum-packaged subprimal cuts after 7 (7 of 9 comparisons), 14 (7 of 9 comparisons) or 21 (8 of 9 comparisons) days of storage, and 1, 3 or 5 days of retail display. The largest decrease in overall appearance ratings for chops stored as retail cuts occurred during the first 7 days of storage.

## DISCUSSION AND CONCLUSIONS

The gas in the headspace of vacuum packages (subprimal cuts, reformed subprimal cuts and retail cuts) was relatively high in percentage of CO<sub>2</sub>. CO<sub>2</sub> has been shown to inhibit aerobic psychrotrophic spoilage bacteria (7,8,10,14) and is responsible, in part, for the success of vacuum packaging (1,5). The percentage of CO<sub>2</sub> usually increased with prolonged storage, concurrent with a decrease in the percentage of O<sub>2</sub>, in the present study.

TABLE 9. Relative weight percentages of carbon dioxide, oxygen and nitrogen in package headspace of lamb during the 3-week storage interval.

| Storage interval (days) | Gas component   | Packaging treatment |                         |                   |                   |                                        |
|-------------------------|-----------------|---------------------|-------------------------|-------------------|-------------------|----------------------------------------|
|                         |                 | Vacuum packaged     |                         | Retail cuts       | Retail cuts       |                                        |
|                         |                 | Subprimal cuts      | Reformed subprimal cuts |                   | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> |
| Initial                 | CO <sub>2</sub> | 1.1 <sup>d</sup>    | 2.1 <sup>d</sup>        | 1.4 <sup>b</sup>  | 16.3 <sup>a</sup> | 28.6 <sup>a</sup>                      |
|                         | O <sub>2</sub>  | 21.7 <sup>a</sup>   | 21.1 <sup>a</sup>       | 21.6 <sup>a</sup> | 4.1 <sup>b</sup>  | 3.9 <sup>b</sup>                       |
|                         | N <sub>2</sub>  | 77.2 <sup>c</sup>   | 76.8 <sup>c</sup>       | 77.1 <sup>b</sup> | 79.6 <sup>a</sup> | 67.4 <sup>b</sup>                      |
| 7                       | CO <sub>2</sub> | 4.5 <sup>c</sup>    | 4.7 <sup>c</sup>        | 1.4 <sup>b</sup>  | 15.1 <sup>a</sup> | 29.3 <sup>a</sup>                      |
|                         | O <sub>2</sub>  | 15.5 <sup>b</sup>   | 16.0 <sup>b</sup>       | 19.1 <sup>b</sup> | 5.8 <sup>b</sup>  | 3.6 <sup>b</sup>                       |
|                         | N <sub>2</sub>  | 80.0 <sup>b</sup>   | 79.3 <sup>bc</sup>      | 76.2 <sup>c</sup> | 79.1 <sup>a</sup> | 67.1 <sup>b</sup>                      |
| 14                      | CO <sub>2</sub> | 6.6 <sup>b</sup>    | 6.8 <sup>b</sup>        | 0.5 <sup>c</sup>  | 13.8 <sup>a</sup> | 28.8 <sup>a</sup>                      |
|                         | O <sub>2</sub>  | 9.7 <sup>c</sup>    | 11.4 <sup>c</sup>       | 21.4 <sup>a</sup> | 6.3 <sup>b</sup>  | 5.1 <sup>b</sup>                       |
|                         | N <sub>2</sub>  | 83.7 <sup>a</sup>   | 81.9 <sup>b</sup>       | 78.1 <sup>a</sup> | 79.9 <sup>a</sup> | 66.1 <sup>b</sup>                      |
| 21                      | CO <sub>2</sub> | 8.9 <sup>a</sup>    | 9.2 <sup>a</sup>        | 8.5 <sup>a</sup>  | 10.9 <sup>b</sup> | 16.7 <sup>b</sup>                      |
|                         | O <sub>2</sub>  | 9.2 <sup>c</sup>    | 2.1 <sup>d</sup>        | 13.4 <sup>c</sup> | 11.4 <sup>a</sup> | 12.8 <sup>a</sup>                      |
|                         | N <sub>2</sub>  | 81.9 <sup>ab</sup>  | 88.8 <sup>a</sup>       | 78.1 <sup>a</sup> | 77.6 <sup>b</sup> | 70.4 <sup>a</sup>                      |

a,b,c,d Means of the same gas component in the same column bearing a common superscript letter do not differ ( $P > .05$ ).

TABLE 10. Mean values for surface discoloration<sup>a</sup> of lamb chops during retail display.

| Day of retail display | Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                  |                  |                                        |
|-----------------------|-------------------------|----------------------------------|-------------------------|------------------|------------------|----------------------------------------|
|                       |                         | Vacuum packaged                  |                         | Retail cuts      | Retail cuts      |                                        |
|                       |                         | Subprimal cuts                   | Reformed subprimal cuts |                  | Vacuum packaged  | 20% CO <sub>2</sub> 80% N <sub>2</sub> |
| 1                     | Initial                 | 5.9 <sup>c</sup>                 | 5.9 <sup>cd</sup>       | 5.9 <sup>c</sup> | 5.9 <sup>c</sup> | 5.9 <sup>c</sup>                       |
|                       | 7                       | 3.9 <sup>d</sup>                 | 5.5 <sup>d</sup>        | 3.1 <sup>d</sup> | 3.2 <sup>d</sup> | 1.9 <sup>d</sup>                       |
|                       | 14                      | 5.1 <sup>e</sup>                 | 6.4 <sup>c</sup>        | 3.4 <sup>d</sup> | 2.2 <sup>e</sup> | 2.3 <sup>d</sup>                       |
|                       | 21                      | 4.4 <sup>e</sup>                 | 6.1 <sup>cd</sup>       | 2.8 <sup>d</sup> | 1.6 <sup>e</sup> | 1.5 <sup>d</sup>                       |
| 3                     | Initial                 | 4.3 <sup>c</sup>                 | 4.3 <sup>d</sup>        | 4.3 <sup>c</sup> | 4.3 <sup>c</sup> | 4.3 <sup>c</sup>                       |
|                       | 7                       | 4.9 <sup>c</sup>                 | 5.9 <sup>c</sup>        | 3.8 <sup>c</sup> | 3.6 <sup>c</sup> | 2.7 <sup>d</sup>                       |
|                       | 14                      | 4.2 <sup>c</sup>                 | 4.7 <sup>d</sup>        | 2.8 <sup>d</sup> | 2.1 <sup>d</sup> | 2.3 <sup>d</sup>                       |
|                       | 21                      | 3.9 <sup>c</sup>                 | 4.7 <sup>d</sup>        | 2.2 <sup>d</sup> | 2.3 <sup>d</sup> | 2.6 <sup>d</sup>                       |
| 5                     | Initial                 | 4.1 <sup>c</sup>                 | 4.1 <sup>cd</sup>       | 4.1 <sup>c</sup> | 4.1 <sup>c</sup> | 4.1 <sup>c</sup>                       |
|                       | 7                       | 3.7 <sup>c</sup>                 | 5.0 <sup>c</sup>        | 3.2 <sup>d</sup> | 2.9 <sup>d</sup> | 2.3 <sup>d</sup>                       |
|                       | 14                      | 3.7 <sup>c</sup>                 | 4.4 <sup>cd</sup>       | 2.8 <sup>d</sup> | 2.4 <sup>d</sup> | 2.2 <sup>d</sup>                       |
|                       | 21                      | 3.5 <sup>c</sup>                 | 3.4 <sup>d</sup>        | 3.1 <sup>d</sup> | 2.4 <sup>d</sup> | 2.4 <sup>d</sup>                       |

<sup>a</sup>Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different ( $P < .05$ ) from means obtained for cuts from vacuum packaged subprimal cuts.

c,d,e Means in the same column and for the same day of retail display bearing a common superscript letter do not differ ( $P > .05$ ).

Respiration by meat tissue and/or bacteria is reported to be responsible for this change (1,5); however, it often appears that a greater percentage of CO<sub>2</sub> occurs than would be possible from respiration. It is possible that some of the CO<sub>2</sub> dissolved in the meat tissue and was released upon exposure after fabrication. Urbin and Wilson (16) also noted CO<sub>2</sub> evolution from post-rigor meat and explained the CO<sub>2</sub> evolution on the basis of pH change and release of bicarbonate in the form of CO<sub>2</sub>. Daun et al. (4) believed that part of the CO<sub>2</sub> increase in modified gas atmosphere packages was a result of interactions of gas solubility, temperature and headspace volume.

Using systems and gas combinations involved in the present study, retail cuts of beef, pork and lamb could not be successfully precut, retail-packaged and stored for 7-21 days in vacuum or modified gas atmospheres. Storage of retail cuts in vacuum packages for 7, 14 or 21 days before display increased ( $P < .05$ ) surface discoloration in 5 of 9 (beef), 7 of 9 (pork) and 6 of 9 (lamb) comparisons and decreased ( $P < .05$ ) overall appearance in 7 of 9 (beef), 4 of 9 (pork) and 6 of 9 (lamb) comparisons with retail cuts from loins stored comparable times in vacuum packages. Storage of retail cuts in modified gas atmospheres (20 or 40% CO<sub>2</sub> and 60 or 80% N<sub>2</sub>) for 7, 14 or 21 days before display increased ( $P < .05$ ) surface discoloration in 13 of 18 (beef), 18 of 18 (pork) and 17 of 18 (lamb) comparisons and decreased ( $P < .05$ ) overall appearance in 14 of 18 (beef), 13 of 18 (pork) and 16 of 18 (lamb) comparisons with retail cuts from loins stored comparable times in vacuum packages.

Conversely, and inexplicably, storage of retail cuts as vacuum-packaged reformed subprimal cuts for 7, 14 or 21 days before display decreased ( $P < .05$ ) surface discoloration in 0 of 9 (beef), 3 of 9 (pork) and 5 of 9 (lamb) comparisons and improved ( $P < .05$ ) overall

appearance in 1 of 9 (beef), 6 of 9 (pork) and 6 of 9 (lamb) comparisons with retail cuts from loins stored comparable times in vacuum packages. Marriott et al. (12) have previously reported very successful attempts to store precut, reformed beef subprimal cuts. Our lack of success in (a) creating adequate vacuum or (b) identifying an appropriate mixture of gases for use in modified gas atmosphere packaging, for storage of retail cuts may have been caused by our attempt to use the 3-shelf cardboard container system. Gas analysis of packages containing retail cuts contained considerably higher concentrations of oxygen than would be desired. Results might have been improved if the rigid cardboard containers had been repeatedly backflushed with modified gas atmospheres so that the atmosphere surrounding the cut would have remained near that in the initial gas mixture. Repeated flushing with CO<sub>2</sub> + N<sub>2</sub> gas mixtures would have reduced the O<sub>2</sub> concentration at meat surfaces thereby reducing discoloration due to metmyoglobin formation. Taylor (15) found that a low concentration of O<sub>2</sub> will result in immediate metmyoglobin formation and brown discoloration, while long-term exposure to any concentration of O<sub>2</sub> usually results in metmyoglobin formation.

Additional research is needed in areas of CO<sub>2</sub> evolution from meat, identification of optimal gas mixtures for modified gas atmosphere packaging, and capitalization on apparent benefits associated with use of precut, reformed subprimal cuts.

#### ACKNOWLEDGMENT

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TABLE 11. Mean values for overall appearance<sup>a</sup> ratings of lamb chops during retail display.

| Day of retail display | Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                  |                                        |                                        |                  |
|-----------------------|-------------------------|----------------------------------|-------------------------|------------------|----------------------------------------|----------------------------------------|------------------|
|                       |                         | Vacuum packaged                  |                         | Retail cuts      |                                        |                                        |                  |
|                       |                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                  |
| 1                     | Initial                 | 6.5 <sup>c</sup>                 | 6.5 <sup>cd</sup>       | 6.5 <sup>c</sup> | 6.5 <sup>c</sup>                       | 6.5 <sup>c</sup>                       | 6.5 <sup>c</sup> |
|                       | 7                       | 4.5 <sup>de</sup>                | 5.9 <sup>d</sup>        | 2.6 <sup>e</sup> | 3.5 <sup>d</sup>                       | 2.6 <sup>d</sup>                       | 2.6 <sup>d</sup> |
|                       | 14                      | 4.1 <sup>d</sup>                 | 7.0 <sup>e</sup>        | 2.9 <sup>d</sup> | 2.8 <sup>e</sup>                       | 2.4 <sup>d</sup>                       | 2.4 <sup>d</sup> |
|                       | 21                      | 3.8 <sup>e</sup>                 | 6.5 <sup>cd</sup>       | 2.1 <sup>e</sup> | 2.0 <sup>f</sup>                       | 2.0 <sup>d</sup>                       | 2.0 <sup>d</sup> |
| 3                     | Initial                 | 4.5 <sup>d</sup>                 | 4.5 <sup>d</sup>        | 4.5 <sup>c</sup> | 4.5 <sup>c</sup>                       | 4.5 <sup>c</sup>                       | 4.5 <sup>c</sup> |
|                       | 7                       | 5.7 <sup>c</sup>                 | 6.7 <sup>c</sup>        | 3.6 <sup>d</sup> | 3.6 <sup>d</sup>                       | 2.7 <sup>d</sup>                       | 2.7 <sup>d</sup> |
|                       | 14                      | 4.2 <sup>d</sup>                 | 5.3 <sup>d</sup>        | 2.3 <sup>d</sup> | 2.7 <sup>d</sup>                       | 2.9 <sup>d</sup>                       | 2.9 <sup>d</sup> |
|                       | 21                      | 3.4 <sup>e</sup>                 | 4.6 <sup>d</sup>        | 2.2 <sup>e</sup> | 2.7 <sup>d</sup>                       | 2.5 <sup>d</sup>                       | 2.5 <sup>d</sup> |
| 5                     | Initial                 | 4.9 <sup>c</sup>                 | 4.9 <sup>cd</sup>       | 4.9 <sup>c</sup> | 4.9 <sup>c</sup>                       | 4.9 <sup>c</sup>                       | 4.9 <sup>c</sup> |
|                       | 7                       | 3.9 <sup>c</sup>                 | 5.5 <sup>c</sup>        | 3.2 <sup>d</sup> | 3.4 <sup>d</sup>                       | 2.2 <sup>d</sup>                       | 2.2 <sup>d</sup> |
|                       | 14                      | 3.5 <sup>c</sup>                 | 5.0 <sup>cd</sup>       | 2.8 <sup>d</sup> | 2.3 <sup>d</sup>                       | 2.7 <sup>d</sup>                       | 2.7 <sup>d</sup> |
|                       | 21                      | 3.4 <sup>c</sup>                 | 3.5 <sup>d</sup>        | 2.7 <sup>d</sup> | 2.4 <sup>d</sup>                       | 2.0 <sup>d</sup>                       | 2.0 <sup>d</sup> |

<sup>a</sup>Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

<sup>b</sup>Means within a common storage interval and day of retail display that are underlined are significantly different ( $P < .05$ ) from means obtained for cuts from vacuum packaged subprimal cuts.

c,d,e,f Means in the same column and for the same day of display bearing a common superscript letter do not differ ( $P > .05$ ).

## Microbiology of Beef, Pork and Lamb Stored in Vacuum or Modified Gas Atmospheres

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### ABSTRACT

Beef, pork and lamb loins were vacuum-packaged as subprimal cuts (controls), reformed subprimal cuts and as retail cuts (steaks or chops). Subprimal cuts, reformed subprimal cuts and one group of retail cuts remained vacuum-packaged. Other groups of retail cuts were injected with atmospheres of either 20% CO<sub>2</sub> + 80% N<sub>2</sub> or 40% CO<sub>2</sub> + 60% N<sub>2</sub>. Cuts were randomly assigned to storage periods of 0-21 days at 2 ± 1 C. At weekly intervals, steaks or chops were removed from each treatment and examined after storage for 5 days under retail display conditions. Psychrotrophic bacterial counts and lactobacilli counts of steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> atmospheres usually were lower, though not often statistically significant, than those of comparable vacuum-packaged steaks, chops or loins. Psychrotrophic counts of steaks and pork chops, initially held in CO<sub>2</sub>-N<sub>2</sub> atmospheres and then subjected to retail display, usually were lower than those of comparable steaks and chops that had been vacuum-packaged (without added CO<sub>2</sub>-N<sub>2</sub>) or prepared from vacuum-packaged loins.

Our current meat packaging research (3,4,17,18) is focussed on reducing quality loss of product and extending shelf life by (a) capitalizing on the economic advantages of centralized breaking and prefabrication, (b) further characterizing the advantages of vacuum packaging and (c) exploring the potential use of new gas atmospheres in meat packaging. The effect of air on microbiological and chemical changes in meat has been investigated extensively. Under normal refrigerated storage, exposure of meat surfaces to air enables aerobic spoilage bacteria to increase and affect color and shelf life. Common aerobic spoilage bacteria such as *Pseudomonas* spp. frequently are responsible for quality losses because of their active proteolytic and lipolytic enzyme systems. Reducing the amount of O<sub>2</sub> inside a package can be accomplished by (a) vacuum packaging or (b) replacing the atmosphere with one that does not contain oxygen (i.e., use of a modified gas atmosphere). The effectiveness of both methods in controlling growth of common aerobic bacteria, and thereby extending shelf life of the product has been demonstrated (2,7,10,15,20). In recent studies (3,4,17,18), a comparison was made of the retail quality of vacuum-packaged meat and of meat packaged in modified gas atmospheres. One of the most effective gas atmospheres for maintaining meat quality in those studies was a mixture of 20% CO<sub>2</sub> and 80% N<sub>2</sub>. Included in current research is an examination of packaging systems for protecting reformed subprimal cuts prepared at a centralized location for distribution to

retail outlets. This paper compares (a) the microbial conditions of vacuum-packaged reformed subprimal cuts, vacuum-packaged retail cuts and modified atmosphere packaged retail cuts of beef, pork and lamb with that of vacuum-packaged subprimal cuts and (b) the effect of these different packaging techniques on the microbial quality of retail steaks and chops during subsequent retail storage.

### MATERIALS AND METHODS

The sources of the beef, pork and lamb loins and preparation of these meats as subprimal cuts, reformed subprimal cuts or as steaks or chops are described by Seideman et al. (19) in a companion paper. Subprimal cuts were packaged at the maximum capacity of the machine (747 mm Hg). Reformed subprimal cuts were prepared by cutting loins into retail cuts (2.5-cm thick), reforming them, followed by vacuum packaging. Retail steaks and chops (2.5-cm thick) were randomly assigned storage treatments for periods of 0, 7, 14 or 21 days at 2 ± 1 C. These cuts were placed on styrofoam trays and overwrapped with oxygen-permeable, polyvinyl chloride film (19). Groups of 3 retail steaks or chops were packaged for retail display and placed in 3-shelf cardboard containers which were then vacuum-packaged. Retail cuts in cardboard containers were assigned, at random, to each of three treatments: (a) one group remained under vacuum, (b) one group was injected with 1,700 cm<sup>3</sup> of a 20% CO<sub>2</sub> + 80% N<sub>2</sub> gas mixture, and (c) one group was injected with 1,700 cm<sup>3</sup> of a 40% CO<sub>2</sub> + 60% N<sub>2</sub> gas mixture. In each of the storage periods, there were 3 steaks or chops per treatment for a total of 12 per atmospheric treatment. All subprimal, reformed subprimal and retail cuts were packaged in polyethylene nylon - surlyn film with the following characteristics: Oxygen Transmission Rate = 69 cc/m<sup>2</sup>/24 h; Carbon Dioxide Transmission Rate = 206 cc/m<sup>2</sup>/24 h. Initially and at the end of each storage interval, comparable packages from each treatment were opened and subjected to microbiological examination. Three steaks or chops from each treatment (Table 1) were examined by swabbing at 12.9 cm<sup>2</sup> (2 in.<sup>2</sup>) area of muscle surface with a dacron swab moistened in 0.1% sterile peptone broth. Psychrotrophic plate counts were made by both the pour- and spread-plate techniques on Plate Count Agar (Difco). Plates were incubated for 10 days at 7 C. A lactobacillus count was made by the pour-plate technique with Lactobacillus MRS broth (Difco) with 1.5% agar added. Plate incubation was at 25 C for 4 days.

In addition, steaks or chops from each of the five treatments were stored for 5 days under simulated retail display conditions (1-3 C, 970 lux of incandescent light). Vacuum-packaged steaks and chops were removed from storage containers and displayed without rewrapping. Steaks or chops from vacuum-packaged subprimal cuts were cut and reformed subprimal cuts were removed from the original package and wrapped with polyvinyl chloride film before retail display. Microbiological analysis of these steaks and chops was as described above.

Analysis of variance comparing data for vacuum-packaged subprimal cuts or data for retail cuts from subprimal cuts stored in

vacuum packages with data from every other treatment was performed within each of the three storage intervals. Comparisons were also made of data within each packaging treatment over the three storage periods. When significant ( $P < .05$ ) main effects were observed in the analysis of variance, mean separation was accomplished by use of the Student-Newman-Keuls test (23).

## RESULTS

Psychrotrophic counts of beef cuts stored at 2 C are presented in Table 2. Differences in counts of subprimal cuts and comparable reformed subprimal and retail cuts were in most instances not statistically significant. Counts of retail cuts which were vacuum-packaged and those packaged in  $\text{CO}_2\text{-N}_2$  after 7 days were numerically lower than the initial counts and were significantly lower than those of comparable vacuum-packaged subprimal cuts. Counts of retail steaks stored in  $\text{CO}_2\text{-N}_2$  atmospheres were lower than those of comparable vacuum-packaged steaks. Retail steaks stored for 14 days in  $\text{CO}_2\text{-N}_2$  had numerically lower counts than comparable subprimal cuts; after 21 days only steaks stored in 40%  $\text{CO}_2\text{-60\% N}_2$  had numerically lower counts than comparable subprimal cuts. After 7 days of storage, counts of reformed subprimal cuts were slightly higher numerically than those of comparable subprimal cuts; they were similar or lower after 14 and 21 days, respectively. The largest increases in count usually occurred between 14 and 21 days of storage. Data

obtained with the pour plate technique (not presented in tabular form) were similar, except that the counts usually were somewhat lower (0.1-0.7 log) than with the spread-plate method.

Differences in lactobacillus counts of subprimal cuts and comparable reformed subprimal and retail cuts were statistically not significant (Table 3). Lactobacillus counts of steaks packaged in  $\text{CO}_2\text{-N}_2$  atmospheres, however, were numerically consistently lower than those of comparable vacuum-packaged subprimal cuts. Lactobacillus counts of steaks stored for 14 and 21 days in  $\text{CO}_2\text{-N}_2$  atmospheres were numerically lower than those of comparable vacuum-packaged steaks. Lactobacillus counts decreased slightly during the first 7 days of storage.

Psychrotrophic counts of beef steaks (derived from the five packaging treatments) after 5 days of retail display are presented in Table 4. Counts of steaks derived from packages stored initially for 7 and 14 days in  $\text{CO}_2\text{-N}_2$  were numerically lower than those of steaks which had been vacuum-packaged for a comparable period. Of the retail steaks initially stored for 21 days in  $\text{CO}_2\text{-N}_2$ , only those stored in 40%  $\text{CO}_2\text{-60\% N}_2$  had, after retail display, counts numerically lower than those of steaks derived from vacuum-packaged steaks. Also, counts of steaks initially stored in  $\text{CO}_2\text{-N}_2$  were, after retail display, usually lower than those of steaks derived from

TABLE 1. Number of samples examined bacteriologically arranged according to packaging treatment and length of storage.

| Storage interval (days) | Packaging treatment |                         |                 |                                    |                                    |
|-------------------------|---------------------|-------------------------|-----------------|------------------------------------|------------------------------------|
|                         | Vacuum packaged     |                         | Vacuum packaged | Retail cuts                        |                                    |
|                         | Subprimal cuts      | Reformed subprimal cuts |                 | 20% $\text{CO}_2$ 80% $\text{N}_2$ | 40% $\text{CO}_2$ 60% $\text{N}_2$ |
| Initial                 |                     |                         | 3 <sup>a</sup>  |                                    |                                    |
| 7                       | 3                   | 3                       | 3               | 3                                  | 3                                  |
| 14                      | 3                   | 3                       | 3               | 3                                  | 3                                  |
| 21                      | 3                   | 3                       | 3               | 3                                  | 3                                  |

<sup>a</sup>Initial counts were obtained on three subprimal cuts and were common to all treatments.

TABLE 2. Mean values for psychrotrophic bacterial counts<sup>a</sup> of beef after storage stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                    |                                    |                                    |
|-------------------------|----------------------------------|-------------------------|--------------------|------------------------------------|------------------------------------|
|                         | Vacuum packaged                  |                         | Vacuum packaged    | Retail cuts                        |                                    |
|                         | Subprimal cuts                   | Reformed subprimal cuts |                    | 20% $\text{CO}_2$ 80% $\text{N}_2$ | 40% $\text{CO}_2$ 60% $\text{N}_2$ |
| Initial                 | 2.79 <sup>d</sup>                | 2.79 <sup>e</sup>       | 2.79 <sup>d</sup>  | 2.79 <sup>e</sup>                  | 2.79 <sup>d</sup>                  |
| 7                       | 2.98 <sup>d</sup>                | 3.37 <sup>e</sup>       | 2.24 <sup>d</sup>  | 2.17 <sup>e</sup>                  | 2.22 <sup>d</sup>                  |
| 14                      | 3.96 <sup>d</sup>                | 3.92 <sup>d</sup>       | 4.89 <sup>cd</sup> | 3.51 <sup>d</sup>                  | 3.12 <sup>d</sup>                  |
| 21                      | 5.02 <sup>c</sup>                | 4.69 <sup>c</sup>       | 6.31 <sup>c</sup>  | 5.07 <sup>c</sup>                  | 4.89 <sup>c</sup>                  |

<sup>a</sup>Counts ( $\log_{10}$ ) per 6.45  $\text{cm}^2$  (1  $\text{in.}^2$ ) (spread-plate method).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different ( $P < .05$ ) from counts obtained from vacuum packaged subprimal cuts.

<sup>cde</sup>Counts in the same column bearing a common superscript letter do not differ ( $P > .05$ ).

TABLE 3. Mean values for lactobacilli counts<sup>a</sup> of beef after storage stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment |                         |                   |                                    |                                    |
|-------------------------|---------------------|-------------------------|-------------------|------------------------------------|------------------------------------|
|                         | Vacuum packaged     |                         | Vacuum packaged   | Retail cuts                        |                                    |
|                         | Subprimal cuts      | Reformed subprimal cuts |                   | 20% $\text{CO}_2$ 80% $\text{N}_2$ | 40% $\text{CO}_2$ 60% $\text{N}_2$ |
| Initial                 | 1.70 <sup>d</sup>   | 1.70 <sup>c</sup>       | 1.70 <sup>d</sup> | 1.70 <sup>d</sup>                  | 1.70 <sup>d</sup>                  |
| 7                       | 1.54 <sup>d</sup>   | 1.45 <sup>c</sup>       | < 1.0             | < 1.0                              | 1.00 <sup>e</sup>                  |
| 14                      | 3.76 <sup>c</sup>   | 2.50 <sup>c</sup>       | 3.56 <sup>c</sup> | 2.57 <sup>c</sup>                  | 2.72 <sup>c</sup>                  |
| 21                      | 5.11 <sup>b</sup>   | 4.72 <sup>b</sup>       | 5.39 <sup>b</sup> | 4.86 <sup>b</sup>                  | 4.34 <sup>b</sup>                  |

<sup>a</sup>Counts ( $\log_{10}$ ) per 6.45  $\text{cm}^2$  (1  $\text{in.}^2$ ).

<sup>bcd</sup>Counts in the same column bearing a common superscript letter do not differ ( $P > .05$ ).

vacuum-packaged subprimal cuts. In three of five instances where counts were lower, the differences in count were statistically significant. Counts of steaks derived from retail cuts initially stored for 21 days (with or without CO<sub>2</sub>-N<sub>2</sub>) were significantly lower than those of steaks derived from vacuum-packaged subprimal cuts. After retail display, counts of steaks derived from reformed subprimal cuts were significantly lower than those from vacuum-packaged subprimal cuts in two of three comparisons. Increases in count during retail display of steaks initially stored in CO<sub>2</sub>-N<sub>2</sub> for 7 or 14 days were smaller than those for cuts initially stored in vacuum packages (Tables 2 and 4). However, increases in count during retail display of steaks initially stored for 21 days in vacuum packages were somewhat lower than those of steaks derived from comparable packages with CO<sub>2</sub>-N<sub>2</sub>.

Psychrotrophic counts of various pork cuts after storage for 0 to 21 days are presented in Table 5. Storage of chops for 7 days in CO<sub>2</sub>-N<sub>2</sub> caused slight numerical decreases in count. Counts of chops stored for 7, 14 and 21 days in CO<sub>2</sub>-N<sub>2</sub> atmospheres were consistently

numerically lower than those of comparable vacuum-packaged chops and usually significantly lower than those of comparable vacuum-packaged subprimal cuts. After storage for 7 or 14 days, counts of reformed subprimal cuts were lower than those of corresponding pork loins; these differences were statistically significant at 14 days. Largest increases in count occurred either between 7 to 14 days of storage (vacuum-packaged loins, vacuum-packaged chops) or between 14 to 21 days (reformed pork loins, chops packaged in CO<sub>2</sub>-N<sub>2</sub>). Data obtained with pour plate counts gave essentially the same information.

After 7, 14 and 21 days of storage, lactobacillus counts of reformed pork loins and those of chops (vacuum-packaged or in CO<sub>2</sub>-N<sub>2</sub>) usually were lower than those of corresponding vacuum-packaged pork loins (Table 6). At 14 days of storage, these differences in count were statistically significant. At 21 days, the differences in lactobacillus count of vacuum-packaged subprimal cuts and retail cuts held in 40% CO<sub>2</sub> + 60% N<sub>2</sub> were statistically significant. Largest increases in lactobacillus counts for the pork loins and reformed pork loins

TABLE 4. Mean values for psychrotrophic bacterial counts<sup>a</sup> of beef steaks after 5 days of retail display.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts       |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| 7                       | 4.63 <sup>d</sup>                | 4.18 <sup>e</sup>       | 5.20 <sup>d</sup> | 3.19 <sup>d</sup>                      | 2.23 <sup>d</sup>                      |
| 14                      | 5.92 <sup>d</sup>                | 5.50 <sup>d</sup>       | 8.49 <sup>c</sup> | 6.77 <sup>c</sup>                      | 5.50 <sup>c</sup>                      |
| 21                      | 8.14 <sup>c</sup>                | 7.23 <sup>c</sup>       | 7.45 <sup>c</sup> | 7.91 <sup>c</sup>                      | 6.35 <sup>c</sup>                      |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>) (spread-plate method).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from steaks from vacuum packaged subprimal cuts.

<sup>cde</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).

TABLE 5. Mean values for psychrotrophic bacterial counts<sup>a</sup> of pork stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts       |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | 2.64 <sup>e</sup>                | 2.64 <sup>e</sup>       | 2.64 <sup>e</sup> | 2.64 <sup>e</sup>                      | 2.64 <sup>cd</sup>                     |
| 7                       | 3.85 <sup>e</sup>                | 2.84 <sup>e</sup>       | 2.93 <sup>e</sup> | 2.43 <sup>e</sup>                      | 2.30 <sup>d</sup>                      |
| 14                      | 5.66 <sup>d</sup>                | 5.15 <sup>d</sup>       | 6.09 <sup>d</sup> | 3.48 <sup>d</sup>                      | 3.39 <sup>cd</sup>                     |
| 21                      | 7.01 <sup>c</sup>                | 7.88 <sup>c</sup>       | 7.33 <sup>c</sup> | 5.72 <sup>c</sup>                      | 5.06 <sup>c</sup>                      |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>) (spread-plate method).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from vacuum packaged subprimal cuts.

<sup>cde</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).

TABLE 6. Mean values for lactobacilli counts<sup>a</sup> of pork stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-------------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts       |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged   | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | 2.49 <sup>d</sup>                | 2.49 <sup>d</sup>       | 2.49 <sup>d</sup> | 2.49 <sup>d</sup>                      | 2.49 <sup>d</sup>                      |
| 7                       | 2.58 <sup>d</sup>                | 2.33 <sup>d</sup>       | 2.98 <sup>d</sup> | 2.64 <sup>d</sup>                      | 2.50 <sup>d</sup>                      |
| 14                      | 5.13 <sup>c</sup>                | 4.14 <sup>c</sup>       | 3.99 <sup>c</sup> | 1.69 <sup>e</sup>                      | 3.03 <sup>d</sup>                      |
| 21                      | 5.79 <sup>c</sup>                | 5.61 <sup>c</sup>       | 5.32 <sup>c</sup> | 5.31 <sup>c</sup>                      | 4.58 <sup>c</sup>                      |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from vacuum packaged subprimal cuts.

<sup>cde</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).

occurred between 7 and 14 days, for the retail chops between 14 and 21 days of storage.

Psychrotrophic counts of pork chops after retail display are presented in Table 7. Pork chops originally stored for 7 days in 20% CO<sub>2</sub> + 80% N<sub>2</sub> and for 7 to 21 days in 40% CO<sub>2</sub> + 60% N<sub>2</sub> had significantly lower counts than did chops prepared from vacuum-packaged pork loins. Counts of chops initially stored in CO<sub>2</sub>-N<sub>2</sub> were, after display, numerically lower than those of chops which had been stored in vacuum packages.

After 7 days of storage, psychrotrophic counts of retail lamb chops stored in CO<sub>2</sub>-N<sub>2</sub> had decreased slightly, although the differences in count were not statistically significant (Table 8). Psychrotrophic counts of lamb chops stored for 7, 14 or 21 days either vacuum-packaged or in CO<sub>2</sub>-N<sub>2</sub> atmospheres frequently were significantly lower than those of corresponding vacuum-packaged lamb loins. Counts of lamb chops stored in CO<sub>2</sub>-N<sub>2</sub> were consistently numerically lower than those of chops that were vacuum-packaged only. Largest increases in count occurred either between 14 and 21 days (vacuum-packaged lamb loins, chops packaged in 40% CO<sub>2</sub> plus 60% N<sub>2</sub>) or between 7 and 14 days (reformed lamb loins,

vacuum-packaged chops and chops stored in 20% CO<sub>2</sub> plus 80% N<sub>2</sub>).

Large increases in lactobacillus counts occurred only between 14 and 21 days of storage (Table 9). In most instances, differences in lactobacillus counts of vacuum-packaged subprimal cuts and reformed subprimal and retail cuts were not statistically significant, except that at 14 days counts of retail chops were significantly higher than those of comparable vacuum-packaged loins. Counts of chops stored in CO<sub>2</sub>-N<sub>2</sub> for 7 and 14 days were numerically slightly lower than those of corresponding vacuum-packaged chops.

Psychrotrophic counts of lamb chops initially stored for 21 days in CO<sub>2</sub>-N<sub>2</sub> were, after retail display, numerically lower than those of chops derived from corresponding vacuum-packaged loins, reformed loins, or from vacuum packaged chops (Table 10). After 7 days counts of chops derived from the five packaging treatments were very similar.

## DISCUSSION

Although differences in counts reported here often were not statistically significant and some exceptions are

TABLE 7. Mean values for psychrotrophic bacterial counts<sup>a</sup> of pork chops after 5 days of retail display.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                         |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-------------------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts             |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged         | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| 7                       | 4.55 <sup>e</sup>                | 4.73 <sup>e</sup>       | <u>6.03<sup>e</sup></u> | <u>4.37<sup>e</sup></u>                | <u>2.73<sup>d</sup></u>                |
| 14                      | 7.25 <sup>d</sup>                | 7.27 <sup>d</sup>       | <u>7.53<sup>d</sup></u> | <u>7.22<sup>d</sup></u>                | <u>5.98<sup>c</sup></u>                |
| 21                      | 8.15 <sup>c</sup>                | 8.82 <sup>c</sup>       | <u>9.12<sup>c</sup></u> | <u>8.50<sup>c</sup></u>                | <u>6.22<sup>c</sup></u>                |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>) (spread-plate method).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from chops from vacuum packaged subprimal cuts.

<sup>cdef</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).

TABLE 8. Mean values for psychrotrophic bacterial counts<sup>a</sup> of lamb after storage stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                         |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-------------------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts             |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged         | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | 2.97 <sup>f</sup>                | 2.97 <sup>e</sup>       | 2.97 <sup>e</sup>       | 2.97 <sup>e</sup>                      | 2.97 <sup>d</sup>                      |
| 7                       | 3.51 <sup>e</sup>                | 3.55 <sup>de</sup>      | 2.96 <sup>e</sup>       | <u>2.54<sup>e</sup></u>                | <u>2.75<sup>d</sup></u>                |
| 14                      | 5.38 <sup>d</sup>                | 5.91 <sup>d</sup>       | <u>6.17<sup>d</sup></u> | <u>4.80<sup>d</sup></u>                | <u>2.60<sup>d</sup></u>                |
| 21                      | 7.75 <sup>c</sup>                | 7.75 <sup>c</sup>       | <u>7.28<sup>c</sup></u> | <u>6.65<sup>c</sup></u>                | <u>5.82<sup>c</sup></u>                |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>) (spread-plate method).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from vacuum packaged subprimal cuts.

<sup>cdef</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).

TABLE 9. Mean values for lactobacilli counts<sup>a</sup> of lamb stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                         |                                        |                                        |
|-------------------------|----------------------------------|-------------------------|-------------------------|----------------------------------------|----------------------------------------|
|                         | Vacuum packaged                  |                         | Retail cuts             |                                        |                                        |
|                         | Subprimal cuts                   | Reformed subprimal cuts | Vacuum packaged         | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | 2.05 <sup>e</sup>                | 2.05 <sup>d</sup>       | 2.05 <sup>f</sup>       | 2.05 <sup>e</sup>                      | 2.05 <sup>e</sup>                      |
| 7                       | 2.64 <sup>d</sup>                | 2.03 <sup>d</sup>       | 2.71 <sup>e</sup>       | 2.48 <sup>e</sup>                      | 2.60 <sup>d</sup>                      |
| 14                      | 2.54 <sup>de</sup>               | 2.58 <sup>d</sup>       | <u>3.58<sup>d</sup></u> | <u>3.12<sup>d</sup></u>                | <u>3.37<sup>d</sup></u>                |
| 21                      | 5.86 <sup>c</sup>                | 6.03 <sup>c</sup>       | <u>5.75<sup>c</sup></u> | <u>5.96<sup>c</sup></u>                | <u>5.96<sup>c</sup></u>                |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from vacuum packaged subprimal cuts.

<sup>cdef</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).



noted, some general observations can be made regarding the psychrotrophic bacterial counts of the various beef, pork and lamb cuts: (a) counts of steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> decreased slightly during the first week of storage, this was also true for vacuum-packaged steaks, (b) counts of steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> were consistently lower than those of comparable vacuum-packaged steaks and chops, (c) compared with the counts of vacuum-packaged loins, counts of steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> were nearly always lower; in 13 of 18 comparisons the differences were statistically significant and (d) counts of vacuum-packaged reformed loins often were numerically lower than those of comparable vacuum-packaged loins.

With respect to lactobacillus counts of the various cuts: (a) counts of vacuum-packaged steaks decreased slightly during the first week of storage, those of pork and lamb increased slightly, (b) counts of steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> were in most instances lower than those of comparable vacuum-packaged steaks or chops, (c) compared with the counts of vacuum packaged loins, counts of steaks or chops stored in CO<sub>2</sub>-N<sub>2</sub> were usually lower, the same was true for vacuum-packaged steaks and pork chops but not for lamb chops and (d) counts of vacuum-packaged reformed loins were usually lower than those of comparable vacuum-packaged loins.

With respect to the psychrotrophic bacterial counts of steaks and chops held under simulated caselife conditions: (a) counts of steaks or pork chops stored initially in CO<sub>2</sub>-N<sub>2</sub> usually were lower than those of vacuum-packaged steaks or chops or those prepared from vacuum-packaged loins, for lamb chops this was true only for chops which initially had been held for 21 days in CO<sub>2</sub>-N<sub>2</sub>, and (b) increases in count of steaks during display were smaller for steaks initially stored in CO<sub>2</sub>-N<sub>2</sub> for 7 or 14 days than for those which were vacuum-packaged; for those initially stored for 21 days in CO<sub>2</sub>-N<sub>2</sub>, increases in count during display were somewhat greater than for those which were vacuum-packaged.

Differences in psychrotrophic bacterial counts between steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> and those of vacuum-packaged steaks, chops and loins are most likely caused by the immediate effect of CO<sub>2</sub> on growth of common gram-negative aerobic bacteria. The inhibitory effect of CO<sub>2</sub> on the aerobic microflora of beef, pork and lamb is clearly demonstrated in reports by Huffman et al. (8), Bala et al. (1), Newton et al. (14) and Silliker et al.

(22). This effect is probably the result of action on decarboxylating enzymes, especially isocitric and malate dehydrogenases (11,12). Although somewhat speculative at the present time, the effect of CO<sub>2</sub> on cell membrane fluidity and hence on its functional properties such as permeability and transport should be considered (5,6). The role of N<sub>2</sub> in the effect of CO<sub>2</sub>-N<sub>2</sub> mixtures on psychrotrophic counts of meat is less clear. Huffman et al. (8) reported that microbial counts of steaks held in N<sub>2</sub> were similar to those of cuts held in air. However, according to Newton et al. (14), counts of lamb chops stored in oxygen-free N<sub>2</sub> were much lower than those of chops stored in air, O<sub>2</sub> + N<sub>2</sub> (80:20) or in air + CO<sub>2</sub> (80:20). Differences in experimental procedures between these studies (related to animal species, method of sampling, agar media, plate incubation conditions, purity of gases) made comparison of data difficult.

The decreases in psychrotrophic counts during the first week of storage of steaks and chops stored in CO<sub>2</sub>-N<sub>2</sub> reflect the effect of CO<sub>2</sub> on the aerobic microflora. When CO<sub>2</sub> is introduced into the package, inhibition can be expected sooner and probably to a greater extent than in comparable vacuum-packaged cuts. Some residual oxygen is still present in the vacuum-packaged steaks, chops and loins and CO<sub>2</sub> will develop gradually because of microbial and muscle tissue enzyme activities. This agrees with the finding that the counts of steaks and chops in CO<sub>2</sub>-N<sub>2</sub> were lower than those of comparable vacuum-packaged steaks, chops and loins.

Previous reports from our laboratory as well as by others (3,4,9,16) have shown that lactic acid bacteria become a significant and often dominant part of the microflora of vacuum-packaged meats and meats stored in gaseous environments which suppress gram-negative aerobic psychrotrophic bacteria. It is difficult to explain why lactobacillus counts of steaks and chops held in CO<sub>2</sub>-N<sub>2</sub> usually were lower than those of comparable vacuum-packaged steaks, chops or loins. In a previous report (3), lactobacillus counts of conventional vacuum-packaged beef roasts also were often somewhat higher than those of roasts stored for 21-35 days in any of six different gas atmospheres. Perhaps the gaseous environment in the vacuum-packaged products was more conducive to development of lactobacilli. Data in the companion paper (19) show that the weight percentages of CO<sub>2</sub> of the vacuum-packaged steaks and chops were lower than those of the steaks and chops stored in

TABLE 10. Mean values for psychrotrophic bacterial counts<sup>a</sup> of lamb chops after 5 days of retail display.

| Storage interval (days) | Packaging treatment <sup>b</sup> |                         |                   |                                           |                                           |
|-------------------------|----------------------------------|-------------------------|-------------------|-------------------------------------------|-------------------------------------------|
|                         | Vacuum packaged                  |                         | Vacuum packaged   | Retail cuts                               |                                           |
|                         | Subprimal cuts                   | Reformed subprimal cuts |                   | 20% CO <sub>2</sub><br>80% N <sub>2</sub> | 40% CO <sub>2</sub><br>60% N <sub>2</sub> |
| 7                       | 6.11 <sup>d</sup>                | 6.18 <sup>c</sup>       | 6.17 <sup>d</sup> | 6.02 <sup>d</sup>                         | 6.05 <sup>d</sup>                         |
| 21                      | 8.72 <sup>c</sup>                | 8.62 <sup>c</sup>       | 9.09 <sup>c</sup> | 8.01 <sup>c</sup>                         | 7.79 <sup>c</sup>                         |

<sup>a</sup>Counts (log<sub>10</sub>) per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>) (spread-plate method).

<sup>b</sup>Counts within a common storage interval that are underlined are significantly different (P < .05) from counts obtained from chops from vacuum packaged subprimal cuts.

<sup>cd</sup>Counts in the same column bearing a common superscript letter do not differ (P > .05).

CO<sub>2</sub>-N<sub>2</sub> atmospheres. On the other hand, Shaw and Nicol (21) reported that neither CO<sub>2</sub> nor O<sub>2</sub> affected lactobacilli.

The effect of CO<sub>2</sub>-N<sub>2</sub> on the psychrotrophic counts of steaks and pork chops, and to a lesser extent on those of lamb chops was still noticeable after simulated retail display for 5 days. Counts of steaks and pork chops initially stored in CO<sub>2</sub>-N<sub>2</sub> were, after display, usually numerically lower than those of comparable vacuum-packaged steaks, pork chops or loins. Also, increases in psychrotrophic counts of steaks during display were smaller for those held in CO<sub>2</sub>-N<sub>2</sub> (for 7 or 14 days) than for those initially stored in vacuum-packages. These observations may reflect differences in the number and types of microorganisms on the samples stored in CO<sub>2</sub>-N<sub>2</sub> versus those held in vacuum packages at the time they were exposed to retail display. No data were collected relative to microbial types. In addition, there may have been, at least for beef steaks (7-14 days in CO<sub>2</sub>-N<sub>2</sub>) and for pork chops (7 days in CO<sub>2</sub>-N<sub>2</sub>), a residual effect of the CO<sub>2</sub>-N<sub>2</sub> mixture on the microbial flora that developed during retail display. At least for CO<sub>2</sub> this effect can take place because of reactivity of this gas with free amino groups of amino acids, peptides, amines and proteins (13). This effect may be different with meats from different species because of differences in physical or chemical characteristics of muscle.

Data presented by Seideman et al. in a companion paper (19) describe surface discoloration and overall appearance of retail beef, pork and lamb samples examined in this study. They reported that prepackaged retail cuts (vacuum-packaged or packaged in CO<sub>2</sub>-N<sub>2</sub>) usually had significantly more surface discoloration and lower overall appearance ratings than retail cuts derived from vacuum-packaged subprimal cuts. Microbiological analyses show that psychrotrophic bacterial counts of steaks and chops prepackaged in CO<sub>2</sub>-N<sub>2</sub> usually were somewhat lower than those of comparable prepackaged vacuum-packaged steaks or chops or vacuum-packaged subprimal cuts. Also, counts of prepackaged vacuum packaged steaks or chops often were higher than those of comparable vacuum-packaged subprimal cuts. Hence, under the present experimental conditions, changes in surface discoloration or overall appearance do not seem to be related to changes in psychrotrophic bacterial counts. It is more likely that the gases, CO<sub>2</sub> and N<sub>2</sub>, had detrimental effects on muscle pigments and thus on color and appearance.

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## Influence of Alpha-Tocopherol (Vitamin E) on Storage Stability of Raw Pork and Bacon

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### ABSTRACT

Feeding supplemental Vitamin E to pigs before slaughter has a beneficial effect on the storage stability of pork during refrigerated and frozen storage, particularly if packaged in non-vacuum packages. The effects of Vitamin E supplementation on bacon are not as pronounced.

Lipid oxidation is one of the major causes of deterioration in the quality of meat and meat products, particularly in frozen stored meats. Undesirable changes in color, flavor and even nutritive value occur as meat fats are oxidized and interact with other meat constituents such as proteins and carbohydrates (2,6).

The fats in meat can be classified as depot or intermuscular fat, and tissue or intramuscular fat. The intramuscular fats exist in close association with protein and contain a high proportion of the total phospholipid content of meat. Although the phospholipid content is small it is highly susceptible to oxidation, which makes it important in influencing meat quality.

Oxidation occurs as a result of the reaction between atmospheric oxygen and the unsaturated fatty acids. Peroxides are intermediate products in the oxidation process, which in turn break down to odor- and flavor-producing compounds. It has been established that the level of lipid peroxides can be significantly reduced in pigs fed supplemental vitamin E (4). Other studies have shown that supplemental vitamin E improves the stability and flavor of veal fat, frozen poultry, milk and pork (3,5,7,8,9,11) when added to the feed over a long period of time.

The objective of this study was to determine if vitamin E supplementation influenced the storage stability of ground pork steak and bacon during refrigerated and frozen storage in vacuum and normal packs.

### MATERIALS AND METHODS

Twenty bacon-weight pigs (weight range 60-70 kg) were allotted at random into two groups of 10. One group received vitamin E (800 mg/day/animal) in their feed for 7 days before slaughtering. Slaughtering was done in the usual manner in a bacon factory. The carcasses were held in the cooler at 2 C for 2 days before removal of the psoas major muscle, or pork steak, from both sides, which were suitably identified.

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All pork steaks from the left hand side were ground individually through 3-mm plate. Each pork steak was divided into vacuum packs and oxygen-permeable packs (oxygen transmission rate of 20,000 cc/m<sup>2</sup>/24 h/atm). All pork steaks from the other side were cured using a brine composed of 18% of salt and 500 ppm of sodium nitrite, then ground and divided into vacuum and normal packs. Samples were then stored at a refrigerator temperature of 5 C and at a freezer temperature of -20 C. The t-test was used to check for statistical significance. The thiobarbituric acid (TBA) test was carried out according to the method of Tarladgis et al. (10).

### RESULTS

Pork from the vitamin E treatment held at 5 C had significantly lower TBA values than those of controls, in both the normal and vacuum packs (Table 1). Pork in the vacuum packs had significantly lower TBA values than those in normal packs. Bacon from the vitamin E treatment held at 5 C showed little significant difference in TBA values from those of controls, in both normal and vacuum packs (Table 2). This may be because bacon contains nitrite which also acts as an antioxidant. Vacuum packaging again significantly reduced the TBA values of the bacon over those of normal packs. A pH value of 6.0 was used as a cut-off point to indicate bacterial spoilage of pork.

During frozen storage the pork from the vitamin E treatment again had significantly lower TBA values when compared with those of controls, in both normal and vacuum packs (Table 3). During frozen storage the bacon produced from the vitamin E treatments showed no significant difference in TBA values over the controls in either the normal or vacuum packs (Table 4). The vacuum packs showed reduced TBA values over the normal packs, particularly for pork during frozen storage.

A final experiment was carried out to observe the effect of cooking on TBA values for both pork and bacon. The mean TBA values of 10 samples tested in each case are summarized in Table 5. An increase in TBA values by a factor of 4 occurred for pork and an increase of less than 2 occurred for bacon.

### DISCUSSION

Vitamin E supplementation has been shown to stabilize animal products from as far back as 1946 (3) and this effect has since been confirmed by many

TABLE 1. *The effects of supplemental vitamin E on the storage stability of pork in normal oxygen-permeable packs and vacuum packs at 5 C.*

| Time               | Control TBA values |       |       | Treatment TBA values |       |       | t. test     |
|--------------------|--------------------|-------|-------|----------------------|-------|-------|-------------|
|                    | pH                 | Mean  | S.D.  | pH                   | Mean  | S.D.  |             |
| <i>Normal pack</i> |                    |       |       |                      |       |       |             |
| 0 day              | 5.55               | 0.338 | 0.023 | 5.7                  | 0.141 | 0.012 | (P < 0.001) |
| 3 day              | 5.6                | 0.937 | 0.717 | 5.7                  | 0.188 | 0.038 | (P < 0.1)   |
| 6 day              | 5.6                | 1.167 | 0.069 | 5.8                  | 0.193 | 0.023 | (P < 0.001) |
| 10 day             | 6.3                | 1.98  | 0.552 | 6.4                  | 0.438 | 0.115 | (P < 0.001) |
| <i>Vacuum pack</i> |                    |       |       |                      |       |       |             |
| 0 week             | 5.6                | 0.241 | 0.037 | 5.8                  | 0.138 | —     | (P < 0.001) |
| 2 week             | 5.6                | 0.266 | 0.015 | 5.8                  | 0.157 | 0.013 | (P < 0.001) |
| 4 week             | 5.6                | 0.434 | 0.059 | 5.8                  | 0.297 | 0.027 | (P < 0.001) |
| 8 week             | 5.6                | 0.513 | 0.036 | 5.8                  | 0.303 | 0.026 | (P < 0.001) |

TABLE 2. *The effects of supplemental vitamin E on the storage stability of bacon in normal oxygen-permeable packs and vacuum packs at 5 C.*

| Time               | Control TBA values |       |       | Treatment TBA values |       |       | t-test      |
|--------------------|--------------------|-------|-------|----------------------|-------|-------|-------------|
|                    | pH                 | Mean  | S.D.  | pH                   | Mean  | S.D.  |             |
| <i>Normal pack</i> |                    |       |       |                      |       |       |             |
| 0 day              | 5.8                | 0.046 | 0.009 | 5.85                 | 0.043 | 0.009 | N.S.        |
| 7 day              | 5.85               | 0.062 | 0.008 | 5.9                  | 0.057 | 0.011 | N.S.        |
| 14 day             | 5.9                | 0.084 | 0.011 | 5.9                  | 0.069 | 0.004 | (P < 0.01)  |
| 30 day             | 5.95               | 0.115 | 0.01  | 6.0                  | 0.117 | 0.015 | N.S.        |
| <i>Vacuum pack</i> |                    |       |       |                      |       |       |             |
| 0 week             | 5.8                | 0.034 | —     | 5.85                 | 0.027 | 0.009 | (P < 0.1)   |
| 12 week            | 5.95               | 0.169 | 0.017 | 6.0                  | 0.089 | 0.067 | (P < 0.001) |
| 14 week            | 5.95               | 0.174 | 0.025 | 6.05                 | 0.114 | 0.01  | (P < 0.01)  |

TABLE 3. *The effect of supplemental vitamin E on the storage stability of pork in normal oxygen-permeable packs and vacuum packs at -20 C.*

| Time               | Control TBA values |       |       | Treatment TBA values |       |       | t-test      |
|--------------------|--------------------|-------|-------|----------------------|-------|-------|-------------|
|                    | pH                 | Mean  | S.D.  | pH                   | Mean  | S.D.  |             |
| <i>Normal pack</i> |                    |       |       |                      |       |       |             |
| 0 week             | 5.6                | 0.341 | 0.021 | 5.7                  | 0.141 | 0.012 | (P < 0.001) |
| 6 week             | 5.6                | 0.510 | 0.209 | 5.75                 | 0.184 | 0.013 | (P < 0.001) |
| 13 week            | 5.6                | 0.746 | 0.240 | 5.8                  | 0.323 | 0.031 | (P < 0.001) |
| <i>Vacuum pack</i> |                    |       |       |                      |       |       |             |
| 0 week             | 5.6                | 0.318 | 0.089 | 5.7                  | 0.138 | —     | (P < 0.001) |
| 7 week             | 5.6                | 0.472 | 0.053 | 5.8                  | 0.304 | 0.015 | (P < 0.001) |
| 16 week            | 5.6                | 0.487 | 0.044 | 5.8                  | 0.282 | 0.026 | (P < 0.001) |

TABLE 4. *The effects of supplemental vitamin E on the storage stability of bacon in normal oxygen-permeable packs and vacuum packs at -20 C.*

| Time               | Control TBA values |       |       | Treatment TBA values |       |       | t-test      |
|--------------------|--------------------|-------|-------|----------------------|-------|-------|-------------|
|                    | pH                 | Mean  | S.D.  | pH                   | Mean  | S.D.  |             |
| <i>Normal pack</i> |                    |       |       |                      |       |       |             |
| 0 week             | 5.9                | 0.044 | 0.009 | 5.9                  | 0.043 | 0.009 | N.S.        |
| 5 week             | 5.9                | 0.093 | 0.009 | 5.9                  | 0.086 | —     | N.S.        |
| 12 week            | 5.9                | 0.156 | 0.015 | 6.0                  | 0.152 | 0.017 | N.S.        |
| <i>Vacuum pack</i> |                    |       |       |                      |       |       |             |
| 0 week             | 5.9                | 0.044 | 0.009 | 5.9                  | 0.043 | 0.009 | N.S.        |
| 5 week             | 5.9                | 0.124 | 0.008 | 5.95                 | 0.103 | —     | (P < 0.001) |
| 19 week            | 5.95               | 0.128 | 0.009 | 6.0                  | 0.156 | 0.018 | N.S.        |

TABLE 5. *Effect of cooking on TBA values for pork and bacon.*

| Meat  | Control  |        |      | Factor | Treatment |        |      |
|-------|----------|--------|------|--------|-----------|--------|------|
|       | Uncooked | Cooked | S.D. |        | Uncooked  | Cooked | S.D. |
| Pork  | 0.338    | 1.31   | —    | 4      | 0.205     | 0.83   | —    |
| Bacon | 0.130    | 0.25   | —    | < 2    | 0.140     | 0.25   | —    |

research workers for various animal products, including poultry and turkey meat (7,8,9), milk (5) and pork (2,6,11). Pig tissue lipids reflect dietary fat which if unsaturated would increase their propensity for oxidative deterioration. While most of the synthetic anti-oxidants are not absorbed, dietary vitamin E is absorbed and when added to pig rations protects against oxidation and extends the keeping quality of pork.

This study shows that vitamin E supplementation reduces the development of rancidity in pork stored at 5 C and -20 C, thereby increasing its shelf life in vacuum packs and oxygen-permeable packs, in particular. Supplementation for a short period before slaughter is sufficient to produce this effect.

The effects of vitamin E supplementation on bacon are less pronounced, probably due to the presence of nitrite which can also act as an antioxidant. However, a recent report (1) from the American Meat Institute states that *a*-tocopherol is beneficial in blocking nitrosamine formation in bacon, and thus further benefits may accrue.

A TBA value of 1.0 would seem to be a good cut-off point for raw pork. On cooking, this value increased to 4.0 at which point a rancid flavor can be detected. This increase in TBA values during cooking is probably associated with denaturation of pigments present with the resultant release of iron which acts as a catalyst for oxidation.

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## Effect of CO<sub>2</sub>-N<sub>2</sub> Atmospheres on the Microbial Flora of Pork

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### ABSTRACT

Pork loins were vacuum-packaged; one group remained vacuum-packaged, other vacuum packages were injected with gas atmospheres of either 20% CO<sub>2</sub> + 80% N<sub>2</sub> or 40% CO<sub>2</sub> + 60% N<sub>2</sub>. Loins were stored for 7, 14, 21 and 28 days at 1-3 C. After each storage interval, chops were prepared from loins and subjected to 5 days of retail display. Psychrotrophic bacterial counts of lean and fat surfaces of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> were frequently significantly lower than counts of comparable sites of vacuum-packaged loins. Lactobacillus counts of the subcutaneous fat cover of loins stored in CO<sub>2</sub> + N<sub>2</sub> were in most instances (7 of 8 comparisons) significantly higher than those of vacuum-packaged loins; this was true in only 2 of 8 comparisons for the lean surface. Psychrotrophic bacterial counts of chops prepared from loins subjected to the various storage treatments were seldom significantly different. During storage of pork loins, lactobacilli became a predominant part of the microflora and comprised more than 90% of the microflora after 21 days for loins stored in CO<sub>2</sub> + N<sub>2</sub> and after 28 days for vacuum-packaged loins. *Pseudomonas* spp. continued to persist to a greater degree in the vacuum-packaged loins than in the loins stored in CO<sub>2</sub> + N<sub>2</sub>.

The inhibitory effect of vacuum packaging and CO<sub>2</sub> on aerobic gram-negative spoilage bacteria is now well documented (1,10,12,13,15,21). King and Nagel (12,13) suggested that CO<sub>2</sub> could affect decarboxylating enzymes such as isocitric and malate dehydrogenases and thus inhibit bacterial growth. In addition, CO<sub>2</sub> perhaps can alter cell membrane fluidity and hence its functional properties such as permeability and transport (6,7). Some investigators suggest storage of meats in atmospheres consisting of carbon dioxide, nitrogen and oxygen to inhibit aerobic spoilage bacteria and prevent metmyoglobin formation (2,24). In theory, oxygen would maintain the presence of oxymyoglobin, whereas carbon dioxide would inhibit growth of psychrotrophic bacteria such as *Pseudomonas* spp. In previous papers (4,5,17,18), we reported on the physical and microbiological characteristics of beef and pork stored in various gas atmospheres including O<sub>2</sub>, CO<sub>2</sub> + O<sub>2</sub>, CO<sub>2</sub> + N<sub>2</sub>, CO<sub>2</sub> + O<sub>2</sub> + N<sub>2</sub> and CO<sub>2</sub> + O<sub>2</sub> + N<sub>2</sub> + CO. Recently (3,20), sensory and microbiological characteristics of retail cuts of beef and pork and lamb initially stored in atmospheres at 20% CO<sub>2</sub> + 80% N<sub>2</sub> and in 40% CO<sub>2</sub> + 60% N<sub>2</sub> and then subjected to retail display were compared with those of cuts prepared from vacuum-packaged loins and subsequently subjected to retail storage. In that study (3,20), retail cuts stored initially in CO<sub>2</sub> + N<sub>2</sub> atmospheres showed more extensive surface discoloration after

retail display than steaks or chops prepared from vacuum-packaged subprimal cuts. Changes in surface discoloration and overall appearance ratings could not be related to changes in microbial counts (3). In a comparison of six different gas atmospheres with conventional vacuum packaging, Seideman et al. (17,18) showed that an atmosphere of 20% CO<sub>2</sub> + 80% N<sub>2</sub> was acceptable for maintaining quality of wholesale cuts of beef and pork. This paper reports on the effect of CO<sub>2</sub>-N<sub>2</sub> atmospheres (20% CO<sub>2</sub> + 80% N<sub>2</sub>, 40% CO<sub>2</sub> + 60% N<sub>2</sub>) as compared with conventional vacuum-packaging on the level and type of microbial flora of pork loins (on lean and subcutaneous fat cover surfaces) stored for 28 days at 1-3 C.

### EXPERIMENTAL

Twenty-five loins from U.S. No. 1 and No. 2 pork carcasses were cut into thirds, randomly paired and then assigned to one of three packaging treatments. All loins were vacuum-packaged by a CVP model A-100 packaging machine in heat-sealable barrier bags as described by Hall et al. (8). One group of loins remained under vacuum (treatment A), one group was injected with 1671 cm<sup>3</sup> of a 20% CO<sub>2</sub> + 80% N<sub>2</sub> gas mixture (treatment B) and one group was injected with 1671 cm<sup>3</sup> of a 40% CO<sub>2</sub> + 80% N<sub>2</sub> gas mixture (treatment C). Bacterial counts were made on three loins before packaging and were used as initial counts for pork loins in all treatments (Table 1). Subprimal cuts from each treatment were randomly assigned to storage periods of 7, 14, 21 or 28 days at 1-3 C.

At the end of each storage period, packages were opened and microbiological analyses then were performed. One choice (2.5 cm in thickness) was removed from the exterior of each subprimal cut, placed in a styrofoam tray and overwrapped with polyvinyl chloride film. These chops were displayed for 5 days under retail conditions (1-3 C with 947-990 lux of incandescent light).

Two sampling procedures were used for microbiological analyses. The longissimus muscle of each subprimal cut was sampled separately by swabbing a 12.9-cm<sup>2</sup> (2 in.<sup>2</sup>) surface area with a sterile dacron swab wetted in 0.1% sterile peptone broth. Each swab was placed in 10 ml of sterile 0.1% peptone broth. The sample jar was shaken and appropriate dilutions were made with sterile 0.1% peptone broth. Psychrotrophic bacterial counts were made by spreading 0.1-ml samples of appropriate dilutions onto plate count agar (Difco) with plate incubation at 7 C for 10 days. Lactobacilli were enumerated by use of Lactobacillus MRS broth (Difco) plus 1.5% agar with plate incubation at 25 C for 4 days.

To sample the subcutaneous fat layer, a sterile cellulose sponge (5.08 × 5.08 × 1.9 cm) was wetted in sterile 0.1% peptone broth. With the aid of a 32.26 cm<sup>2</sup> (5 in.<sup>2</sup>) aluminum template, a 64.52 cm<sup>2</sup> area of the two loins was sampled by swabbing a 32.26 cm<sup>2</sup> area of one loin and a 32.26 cm<sup>2</sup> area of the other loin. The sponge was then placed in 100 ml of sterile 0.1% peptone broth and squeezed several times in the broth. Preparation of dilutions, plating and incubation was as described for the longissimus muscle.

Psychrotrophic counts of chops after 5 days of retail storage were made in the manner described for the longissimus muscle. Distribution of the psychrotrophic microbial flora of the longissimus muscle after each storage period was determined by picking 30-40 colonies at random from countable plates and placing them on trypticase soy agar (BBL) slants. Incubation of slants was at 25 C for 2-3 days. Diagnostic schemes and procedures for identification of the isolates are those published by Vanderzant and Nickelson (25).

Bacteriological count data were analyzed using analysis of variance. When significant ( $P < .05$ ) main effects were observed, mean separation was accomplished by use of the Student-Newman-Keuls test (11).

## RESULTS

Psychrotrophic bacterial counts of the lean and of the subcutaneous fat cover of pork loins are presented in Tables 2 and 3, respectively. Counts of the lean and subcutaneous fat cover of loins stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> were, in 7 of 8 comparisons higher ( $P < .05$ ) than counts of comparable loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub>. Compared with counts of corresponding vacuum-packaged loins, counts of loins stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> were higher ( $P < .05$ ) in only 2 of 8 comparisons. Counts of the lean and subcutaneous fat cover of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> were in 5 of 8 comparisons significantly lower and in all instances numerically lower than counts of comparable vacuum packaged loins. After 7 to 28 days of storage, counts of the lean surface were nearly always higher than those of the subcutaneous fat cover of the same loins. During a 28-day storage period, counts of the lean of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> increased 3.2 logs, for those stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> and in vacuum-packaged the increases amounted to 3.91 and 4.77 logs, respectively. Similar increases in count of the subcutaneous fat cover of the same loins were 2.79, 4.04 and 3.21 logs, respectively.

Lactobacillus counts of the lean and of the subcutaneous fat cover of pork loins are presented in Tables 4 and 5, respectively. Lactobacillus counts of the lean surfaces were numerically higher than those of the subcutaneous fat cover of the same loins. Counts of the subcutaneous fat cover of loins stored in CO<sub>2</sub> + N<sub>2</sub> were in most instances significantly higher than those of vacuum-packaged loins; those of the lean surface of loins stored in CO<sub>2</sub> + N<sub>2</sub> were, in only 2 of 8 comparisons higher ( $P < .05$ ) than those of vacuum-packaged loins. During the 28-day storage period, lactobacillus counts of

TABLE 2. Psychrotrophic bacterial counts of lean of pork loins stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                                        |                                        | Order of means <sup>b</sup> |
|-------------------------|----------------------------------|----------------------------------------|----------------------------------------|-----------------------------|
|                         | A                                | B                                      | C                                      |                             |
|                         | Vacuum packaged                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                             |
| 0                       | 2.99 <sup>e</sup>                | 2.99 <sup>e</sup>                      | 2.99 <sup>e</sup>                      | ABC                         |
| 7                       | 5.18 <sup>d</sup>                | 4.98 <sup>d</sup>                      | 4.88 <sup>d</sup>                      | ABC                         |
| 14                      | 5.31 <sup>d</sup>                | 5.61 <sup>c</sup>                      | 3.92 <sup>d</sup>                      | BA C                        |
| 21                      | 5.82 <sup>d</sup>                | 7.28 <sup>c</sup>                      | 5.22 <sup>c</sup>                      | B AC                        |
| 28                      | 7.76 <sup>c</sup>                | 6.90 <sup>c</sup>                      | 6.19 <sup>c</sup>                      | A B C                       |

<sup>a</sup>Counts log<sub>10</sub> per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Means in the same row underscored by a common line do not differ ( $P > .05$ ).

<sup>cde</sup>Means in the same column bearing a common superscript letter do not differ ( $P > .05$ ).

TABLE 3. Psychrotrophic bacterial counts of subcutaneous fat cover of pork loins stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                                        |                                        | Order of means <sup>b</sup> |
|-------------------------|----------------------------------|----------------------------------------|----------------------------------------|-----------------------------|
|                         | A                                | B                                      | C                                      |                             |
|                         | Vacuum packaged                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                             |
| 0                       | 3.26 <sup>e</sup>                | 3.26 <sup>e</sup>                      | 3.26 <sup>e</sup>                      | ABC                         |
| 7                       | 4.18 <sup>de</sup>               | 4.12 <sup>de</sup>                     | 2.51 <sup>f</sup>                      | AB C                        |
| 14                      | 4.34 <sup>d</sup>                | 4.68 <sup>d</sup>                      | 3.81 <sup>e</sup>                      | BA C                        |
| 21                      | 5.63 <sup>c</sup>                | 5.88 <sup>d</sup>                      | 5.05 <sup>d</sup>                      | BA C                        |
| 28                      | 6.47 <sup>c</sup>                | 7.30 <sup>c</sup>                      | 6.05 <sup>c</sup>                      | B AC                        |

<sup>a</sup>Counts log<sub>10</sub> per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Means in the same row underscored by a common line do not differ ( $P > .05$ ).

<sup>cdef</sup>Means in the same column having a common superscript letter do not differ ( $P > .05$ ).

TABLE 4. Lactobacillus counts of lean of pork loins stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                                        |                                        | Order of means <sup>b</sup> |
|-------------------------|----------------------------------|----------------------------------------|----------------------------------------|-----------------------------|
|                         | A                                | B                                      | C                                      |                             |
|                         | Vacuum packaged                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                             |
| 0                       | 1.00 <sup>e</sup>                | 1.00 <sup>f</sup>                      | 1.00 <sup>e</sup>                      | ABC                         |
| 7                       | 1.54 <sup>e</sup>                | 2.38 <sup>e</sup>                      | 1.52 <sup>e</sup>                      | BAC                         |
| 14                      | 3.61 <sup>d</sup>                | 3.14 <sup>d</sup>                      | 2.18 <sup>d</sup>                      | AB C                        |
| 21                      | 3.05 <sup>d</sup>                | 4.49 <sup>c</sup>                      | 4.34 <sup>c</sup>                      | BC A                        |
| 28                      | 5.20 <sup>c</sup>                | 5.57 <sup>c</sup>                      | 5.19 <sup>c</sup>                      | BAC                         |

<sup>a</sup>Counts log<sub>10</sub> per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Means in the same row underscored by a common line do not differ ( $P > .05$ ).

<sup>cdef</sup>Means in the same column bearing a common superscript letter do not differ ( $P > .05$ ).

TABLE 1. Number of samples examined bacteriologically arranged according to packaging treatment and length of storage.

| Storage interval (days) | Packaging treatment |               |                                          |               |                                          |               |
|-------------------------|---------------------|---------------|------------------------------------------|---------------|------------------------------------------|---------------|
|                         | A                   |               | B                                        |               | C                                        |               |
|                         | Vacuum packaged     |               | 20% CO <sub>2</sub> + 80% N <sub>2</sub> |               | 40% CO <sub>2</sub> + 60% N <sub>2</sub> |               |
|                         | No. of pkgs.        | No. of subpr. | No. of pkgs.                             | No. of subpr. | No. of pkgs.                             | No. of subpr. |
| Initial                 |                     |               |                                          |               |                                          |               |
| 7                       | 3                   | 6             | 3                                        | 6             | 3                                        | 6             |
| 14                      | 3                   | 6             | 3                                        | 6             | 3                                        | 6             |
| 21                      | 3                   | 6             | 3                                        | 6             | 3                                        | 6             |
| 28                      | 3                   | 6             | 3                                        | 6             | 3                                        | 6             |

<sup>a</sup>Initial counts (day 0) were obtained on 3 subprimal cuts and were common to all treatments.

TABLE 5. *Lactobacillus* counts of subcutaneous fat cover of pork loins stratified according to packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                                        |                                        | Order of means <sup>b</sup> |
|-------------------------|----------------------------------|----------------------------------------|----------------------------------------|-----------------------------|
|                         | A                                | B                                      | C                                      |                             |
|                         | Vacuum packaged                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                             |
| 0                       | 0.44 <sup>c</sup>                | 0.44 <sup>c</sup>                      | 0.44 <sup>c</sup>                      | ABC                         |
| 7                       | 0.98 <sup>e</sup>                | 1.26 <sup>e</sup>                      | 1.36 <sup>d</sup>                      | CB A                        |
| 14                      | 0.99 <sup>e</sup>                | 2.07 <sup>d</sup>                      | 1.96 <sup>d</sup>                      | B C A                       |
| 21                      | 1.59 <sup>d</sup>                | 4.04 <sup>c</sup>                      | 2.18 <sup>d</sup>                      | B C A                       |
| 28                      | 4.66 <sup>c</sup>                | 4.78 <sup>c</sup>                      | 5.13 <sup>c</sup>                      | C BA                        |

<sup>a</sup>Counts log<sub>10</sub> per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Means in the same row underscored by a common line do not differ (P > .05).

<sup>cde</sup>Means in the same column bearing a common superscript letter do not differ (P > .05).

the lean surface of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> increased 4.19 logs, for those stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> and in vacuum packages the increases were 4.57 and 4.20 logs, respectively. Similar increases in *Lactobacillus* count of the subcutaneous fat cover of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub>, 20% CO<sub>2</sub> + 80% N<sub>2</sub> and vacuum packages were 4.69, 4.34 and 4.24 logs, respectively.

Mean values for psychrotrophic bacterial counts of retail pork chops (obtained from loins in each of the packaging and storage treatments) after 5 days of retail display are presented in Table 6. Compared with chops prepared from vacuum packaged loins, counts of chops from loins stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> usually were numerically slightly higher, whereas counts of chops prepared from loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> usually were slightly lower; however, few of these differences were statistically significant. Counts of chops prepared from loins stored initially for 7 days either vacuum-packaged or in CO<sub>2</sub> + N<sub>2</sub> were, after 5 days of retail display slightly lower than the counts of loins from which they had been prepared (Table 2 and 6). However, counts of chops prepared from loins stored for 14-28 days were nearly always somewhat higher than the counts of the loins from which they had been fabricated.

The distribution of the psychrotrophic microbial flora of the lean surface of pork loins stored in vacuum packages or in CO<sub>2</sub> + N<sub>2</sub> is presented in Table 7. The microbial flora of the pork immediately before packaging was dominated by *Pseudomonas* spp. (87.8%). As storage progressed, the proportion of *Pseudomonas* spp. in the microflora of pork loins decreased and that of the lactobacilli increased. After 7 and 14 days of storage, the reduction in percentage of *Pseudomonas* spp. and concomitant increase in lactobacilli was greatest on loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub>. After 21 and 28 days, *Pseudomonas* spp. continued to be a more substantial part (20.5 to 5.4%) of the microflora of vacuum packaged loins than of those stored in CO<sub>2</sub>-N<sub>2</sub> (0-1.2%).

#### DISCUSSION

Psychrotrophic counts of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> were lower than those stored in 20% CO<sub>2</sub> + 80% N<sub>2</sub> or in vacuum packages probably because of the

TABLE 6. Psychrotrophic bacterial counts of lean of pork chops after 5 days of retail display stratified according to pork loin packaging treatment and storage interval.

| Storage interval (days) | Packaging treatment <sup>a</sup> |                                        |                                        | Order of means <sup>b</sup> |
|-------------------------|----------------------------------|----------------------------------------|----------------------------------------|-----------------------------|
|                         | A                                | B                                      | C                                      |                             |
|                         | Vacuum packaged                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |                             |
| 0                       | 5.55 <sup>de</sup>               | 5.55 <sup>de</sup>                     | 5.55 <sup>de</sup>                     | ABC                         |
| 7                       | 5.04 <sup>e</sup>                | 4.53 <sup>e</sup>                      | 4.79 <sup>e</sup>                      | ACB                         |
| 14                      | 6.27 <sup>d</sup>                | 6.59 <sup>d</sup>                      | 5.38 <sup>d</sup>                      | B CA                        |
| 21                      | 6.23 <sup>d</sup>                | 6.81 <sup>d</sup>                      | 6.42 <sup>d</sup>                      | BCA                         |
| 28                      | 8.22 <sup>c</sup>                | 8.52 <sup>c</sup>                      | 7.99 <sup>c</sup>                      | BAC                         |

<sup>a</sup>Counts log<sub>10</sub> per 6.45 cm<sup>2</sup> (1 in.<sup>2</sup>).

<sup>b</sup>Means in the same row underscored by a common line do not differ (P > .05).

<sup>cde</sup>Means in the same column bearing a common superscript letter do not differ (P > .05).

TABLE 7. Percentage distribution of microorganisms on lean of pork loins stratified according to packaging treatment and storage interval.

| Storage interval (days) | Microbial type       | Packaging treatment <sup>a</sup> |                                        |                                        |
|-------------------------|----------------------|----------------------------------|----------------------------------------|----------------------------------------|
|                         |                      | Vacuum packaged                  | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| 7                       | <i>Pseudomonas</i>   | -----                            | 71.8                                   | 43.8                                   |
|                         | <i>Lactobacillus</i> | -----                            | 22.9                                   | 39.8                                   |
|                         | Other <sup>b</sup>   | -----                            | 5.3                                    | 16.4                                   |
| 14                      | <i>Pseudomonas</i>   | 39.2                             | 11.8                                   | 1.8                                    |
|                         | <i>Lactobacillus</i> | 52.2                             | 61.9                                   | 80.1                                   |
|                         | Other <sup>b</sup>   | 8.6                              | 26.3                                   | 18.1                                   |
| 21                      | <i>Pseudomonas</i>   | 20.5                             | 1.2                                    | 0.9                                    |
|                         | <i>Lactobacillus</i> | 74.9                             | 97.5                                   | 94.8                                   |
|                         | Other <sup>b</sup>   | 4.6                              | 1.3                                    | 4.3                                    |
| 28                      | <i>Pseudomonas</i>   | 5.4                              | 0.8                                    | 0                                      |
|                         | <i>Lactobacillus</i> | 92.8                             | 99.2                                   | 95.0                                   |
|                         | Other <sup>b</sup>   | 1.8                              | 0                                      | 5.0                                    |

<sup>a</sup>Each percentage is based on 3 analyses. (Initial flora: *Pseudomonas* 87.8%, *Lactobacillus* 10.6%, Other 1.6%).

<sup>b</sup>These included *Flavobacterium*, *Moraxella-Acinetobacter*, *Leuconostoc*, *Corynebacterium*, *Micrococcus*, *Microbacterium*, *Enterobacter*, Yeast and mold.

immediate presence of inhibiting concentrations of CO<sub>2</sub> and a more limiting concentration of O<sub>2</sub>. Data in the companion paper (8) show that the relative weight percentages of CO<sub>2</sub> in the headspace of the packages injected with 40% CO<sub>2</sub> + 60% N<sub>2</sub> ranged from 22.78 to 31.16%. The relative weight percentages of CO<sub>2</sub> in the headspace of vacuum-packaged loins did not reach 20% until after 14 days of storage. This level (20%) of CO<sub>2</sub> was reached more gradually in the vacuum packages because it resulted from continued activity of meat tissue enzymes and microbial metabolism. Although the inhibitory effect of CO<sub>2</sub> on gram-negative, aerobic psychrotrophic meat bacteria is now well established (1,12,13,14), the role of N<sub>2</sub> on the microflora of meat is still not clear. Huffman (9) reported that aerobic plate counts of pork chops stored in N<sub>2</sub> were similar to those stored in air. However, according to Newton et al. (14), counts of lamb chops stored in N<sub>2</sub> (O<sub>2</sub>-free) were much lower than those of chops stored in air, 80% O<sub>2</sub> + 20% N<sub>2</sub>, or in 80%



air + 20% CO<sub>2</sub>. Differences in the nature of the samples, plating media, conditions of plate incubation, sampling methods and compositions of the gases may have been responsible for the lack of agreement.

The development and subsequent predominance of lactic acid bacteria on vacuum-packaged meat or on meat stored in atmospheres which suppress aerobic psychrotrophic bacteria is well documented (9,14,15,16,19). According to Shaw and Nicol (22), lactobacilli are not affected by either CO<sub>2</sub> or O<sub>2</sub>. In this study, lactobacillus counts of the lean surface of pork loins were numerically higher than those of the subcutaneous fat cover of the same loins. This may have been caused in part by the higher (0.56 log) initial lactobacillus count of the lean surface. No specific reason can be given for the higher lactobacillus counts of the subcutaneous fat cover of loins stored in CO<sub>2</sub> + N<sub>2</sub> as compared to those of vacuum-packaged loins.

Psychrotrophic bacterial counts of chops prepared from loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> were, after retail display, not significantly different from those of chops prepared from vacuum-packaged loins, although the counts of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> were either numerically (4 of 4 comparisons) or significantly lower (2 of 4 comparisons) than those of vacuum-packaged loins following storage. This change in the relation of counts between loins and chops occurred because increases in count of chops during retail display were nearly always greater (1.2-1.8 logs) for chops from loins initially stored for 14-28 days in 40% CO<sub>2</sub> + 60% N<sub>2</sub> than for those (0.41-0.96 logs) from vacuum-packaged loins. On the other hand, Silliker et al. (23) reported a residual inhibitory effect of CO<sub>2</sub> on pork and beef during posttreatment storage in air.

During storage of pork loins in vacuum packages or in CO<sub>2</sub> + N<sub>2</sub> atmospheres, there were marked decreases in the percentage of *Pseudomonas* spp. and increases in the percentage of lactobacilli in the microflora. After 28 days of storage, lactobacilli were predominant (92.8-99.2%) in the microflora of all loins. These data agree with previous reports (9,14,15,19) on the microflora of beef and pork stored in vacuum packages or in CO<sub>2</sub> + N<sub>2</sub> atmospheres. They are also supported by data in the companion paper (8) on the relative weight percentages of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in the headspace gas of the packages. The sharp decrease in the percentage of *Pseudomonas* spp. in the microflora of loins stored for only 7 days in packages injected with 40% CO<sub>2</sub> + 60% N<sub>2</sub>, undoubtedly was caused by the immediate presence of a relatively high concentration of CO<sub>2</sub> which had an inhibitory effect on aerobic psychrotrophic bacteria such as *Pseudomonas* spp. In addition, Shaw and Nicol (22) reported that *Pseudomonas* spp. were affected by O<sub>2</sub> concentrations below 0.8%.

In the companion paper, Hall et al. (8) evaluated loins subjected to the three packaging treatments for muscle color, fat cover appearance, off-odor, muscle surface discoloration and overall appearance. Retail cuts

prepared from these loins were evaluated during a 5-day retail display for surface discoloration, overall appearance and sensory characteristics (juiciness, flavor, overall palatability). Few significant differences were found in appearance or palatability characteristics of loins or chops subjected to the three packaging treatments (8). Microbiological data also indicated few significant differences in aerobic plate counts of chops prepared from loins subjected to the different packaging treatments. Aerobic plate counts of loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub> often were significantly lower than those of vacuum-packaged loins. Hence one might have expected some favorable effect on the sensory characteristics of the loins stored in 40% CO<sub>2</sub> + 60% N<sub>2</sub>. This did not occur (8). On the other hand, the number of viable cells on the loins did not reach the level at which organoleptic defects would be expected until loins had been stored for 28 days.

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## Physical and Sensory Characteristics of Pork Loins Stored in Vacuum or Modified Atmosphere Packages

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### ABSTRACT

Wholesale pork loins were either vacuum-packaged, packaged in an atmosphere of 20% CO<sub>2</sub> + 80% N<sub>2</sub> or packaged in an atmosphere of 40% CO<sub>2</sub> + 60% N<sub>2</sub>. After storage, the primal cuts were evaluated visually for muscle color, fat cover appearance, off-odor, lean surface discoloration and overall appearance. Retail cuts fabricated from the wholesale loins were evaluated for surface discoloration and overall appearance during a 5-day retail display period or they were used for sensory panel evaluation. Few significant differences between packaging treatments were found during the storage period for traits evaluated on the primal cuts. Few significant differences between packaging treatments were observed for either retail display data or sensory panel evaluation.

In recent years vacuum packaging has played a prominent role in distribution of fresh meats. Vacuum-packaging has been reported to extend the effective storage life of fresh meats (1,3,9,14); however, some disadvantages for vacuum-packaging have also been reported. Economic losses associated with purge and distortion of cuts due to vacuum-packaging have been described (3). The color of vacuum-packaged meat is that of reduced myoglobin (3), which has generally been considered unacceptable to the consumer. Physical appearance and color of retail cuts are two of the most important attributes that consumers use to select and purchase meat (6,7,8,10).

Because of problems associated with vacuum-packaging and with consumer acceptance of the muscle color of vacuum-packaged meat, the need exists to search for packaging alternatives which might eliminate these problems. The present study compared vacuum-packaging with modified gas atmosphere-packaging systems which differed in percentages of CO<sub>2</sub> and N<sub>2</sub> and determined the effects of vacuum-packaging and modified gas atmosphere-packaging on the physical appearance of primal and retail cuts of pork, as well as

the palatability characteristics of retail pork cuts. The microbiological aspects of this study are presented in a companion paper by Christopher et al. (4).

### MATERIALS AND METHODS

#### Selection of gases

One gas combination, 20% CO<sub>2</sub> + 80% N<sub>2</sub>, was selected because this concentration of CO<sub>2</sub> is often credited to have some inhibitory effect on aerobic psychrotrophic meat spoilage bacteria (13). A second atmosphere, 40% CO<sub>2</sub> + 60% N<sub>2</sub> was chosen to determine the effects of doubling the initial concentration of carbon dioxide. Both atmospheres were compared to vacuum-packaging with respect to maintenance of the quality (appearance and palatability) of pork cuts.

#### Selection and fabrication of cuts

Ninety subprimal cuts were obtained from wholesale loins of U.S. No. 1 and No. 2 pork carcasses.

#### Packaging treatments

Cuts were randomly paired, weighed and pairs of cuts were assigned to one of three packaging treatments: (a) vacuum-packaged, (b) packaged in an atmosphere of 20% CO<sub>2</sub> + 80% N<sub>2</sub> or (c) packaged in an atmosphere of 40% CO<sub>2</sub> + 60% N<sub>2</sub>. Thus a total of 15 bags, containing two subprimal cuts each, were prepared for each treatment. Each treatment was further subdivided into five storage intervals (0, 7, 14, 21 or 28 days) as shown in the experimental design (Table 1).

All cuts were packaged using a CVP (model A-100) packaging machine capable of either vacuum- or atmospheric-packaging. A vacuum pressure of 635 mm of mercury was used for all packaging. Cuts were placed in polyethylene-nylon-surlin bags (Oxygen Transmission Rate, OTR = 69 cc/m<sup>2</sup>/24 h; Carbon dioxide Transmission Rate, CO<sub>2</sub>TR = 206 cc/m<sup>2</sup>/24 h). Cuts assigned to the vacuum-packaged treatment were hermetically sealed after vacuumization. Packages assigned to an atmospheric treatment were vacuumized and then back-flushed (injected) with 1671 cm<sup>3</sup> of the appropriate gas mixture before sealing. All packages were placed in cardboard cartons and stored at 1-3 C for the appropriate storage interval.

#### Gas analysis

The relative weight percentages of oxygen, nitrogen and carbon dioxide were determined in the headspace of packaged meats. These analyses were repeated at weekly intervals during the 28-day storage period. Silicone glue was applied to a spot on the exterior of the

TABLE 1. Experimental design for the storage of pork loins.

| Storage interval (days) | Packaging treatment |               |                                                |               |                                                |               |
|-------------------------|---------------------|---------------|------------------------------------------------|---------------|------------------------------------------------|---------------|
|                         | Vacuum packaged     |               | 20% CO <sub>2</sub> + 80% N <sub>2</sub> Atmo. |               | 40% CO <sub>2</sub> + 60% N <sub>2</sub> Atmo. |               |
|                         | No. of pkgs.        | No. of subpr. | No. of pkgs.                                   | No. of subpr. | No. of pkgs.                                   | No. of subpr. |
| 0                       | 3                   | 6             | 3                                              | 6             | 3                                              | 6             |
| 7                       | 3                   | 6             | 3                                              | 6             | 3                                              | 6             |
| 14                      | 3                   | 6             | 3                                              | 6             | 3                                              | 6             |
| 21                      | 3                   | 6             | 3                                              | 6             | 3                                              | 6             |
| 28                      | 3                   | 6             | 3                                              | 6             | 3                                              | 6             |

packaging film and allowed to dry to facilitate sampling of the headspace atmospheres. This provided a sampling area that would not tear when punctured and would reseal itself after sampling through an 18-gauge needle. The headspace gas was flushed through a 5-ml sampling loop for 30 sec, at a flow rate of 60 ml/min, at which time the contents of the sampling loop were flushed onto the gas chromatographic column. Fifty ml of helium were injected into vacuum packages at the termination of each storage period to create sufficient gas volume for sampling. Packages were equilibrated at  $2 \pm 1$  C for 10 min before sampling the headspace gases.

The permanent gases were resolved in a Gow-Mac series 550 gas chromatograph fitted with two columns (1.52m  $\times$  6.35 mm) in series and operated at a helium flow rate of 50 ml/min. The first column was packed with 80-100 mesh Porapak-Q and the second column with 80-100 mesh Molecular Sieve 5A. The column oven was operated at 60 C and the detector was set at 100 C. The thermal conductivity detector was operated at a bridge current of 130 ma. Gas chromatographic results were quantitated with a Hewlett-Packard Model 3380A integrator, which was calibrated with a standard gas mixture (Airco) consisting of 50% CO<sub>2</sub>, 10% O<sub>2</sub> and 40% N<sub>2</sub>.

#### Subprimal cut evaluation

Following a 30-min blooming period, subprimal cuts were subjectively evaluated by two trained evaluators for muscle color, using a 9-point scale (9 = very light cherry red, 1 = very dark purple); off-odor, using a 4-point scale (4 = no detectable off-odor, 1 = extreme off-odor); fat cover appearance, using a 7-point scale (7 = no surface discoloration, 1 = total surface discoloration); muscle surface discoloration, utilizing a 7-point scale (7 = no surface discoloration, 1 = total surface discoloration); and overall appearance using an 8-point scale (8 = extremely desirable, 1 = extremely undesirable).

#### Retail cut evaluation

After subprimal cuts were evaluated, a 1.25-cm slice was cut off and discarded to remove discolored lean from exposed faces and two chops (2.5-cm thick) were removed from each subprimal cut. One chop was wrapped and frozen for subsequent cooking and sensory panel evaluation. The second chop was placed on a styrofoam tray, overwrapped with an oxygen-permeable, polyvinyl chloride film and displayed under simulated retail case conditions (1-3 C, 947-990 lux of incandescent light). Chops were evaluated at 24-h intervals by members of a trained evaluation panel during a 5-day retail display period. Chops were evaluated for surface discoloration, employing a 7-point scale (7 = no surface discoloration, 1 = total surface discoloration) and overall appearance using an 8-point scale (8 = extremely desirable, 1 = extremely undesirable).

#### Sensory panel evaluation

Thawed (10 C for 24 h) chops were cooked to an internal temperature of 75 C in a 177-C electric oven. Each chop was cut into 2.5-cm cubes and cubes were evaluated by an 8-member, trained sensory panel for juiciness, using an 8-point scale (8 = extremely juicy, 1 = extremely dry); flavor, using an 8-point scale (8 = extremely desirable, 1 = extremely undesirable); and overall palatability, employing an 8-point scale (8 = extremely desirable, 1 = extremely undesirable). Cuts with coded identity were evaluated in complete random sequence; evaluators had no knowledge regarding treatment of a sample when they evaluated it.

#### Statistical analysis

All data were analyzed using analysis of variance. When significant ( $P < .05$ ) main effects were observed, mean separation was accomplished using the Kramer modification of the Student-Newman-Keuls test (15).

## RESULTS AND DISCUSSION

#### Gas analysis

Relative weight percentages of CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> in the gas sample withdrawn from the headspace of packages in each treatment are presented in Table 2. Relative

TABLE 2. Relative weight percentages of CO<sub>2</sub>, O<sub>2</sub>, and N<sub>2</sub> from packages containing pork loins stratified according to packaging treatment and storage interval.

| Storage interval (days) | Gas             | Packaging treatment |                                        |                                        |
|-------------------------|-----------------|---------------------|----------------------------------------|----------------------------------------|
|                         |                 | Vacuum packaged     | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Initial                 | CO <sub>2</sub> | 1.67 <sup>b</sup>   | 15.48 <sup>a</sup>                     | 31.16 <sup>a</sup>                     |
|                         | O <sub>2</sub>  | 21.59 <sup>a</sup>  | 7.49 <sup>a</sup>                      | 4.71 <sup>a</sup>                      |
|                         | N <sub>2</sub>  | 76.74 <sup>a</sup>  | 77.04 <sup>b</sup>                     | 64.12 <sup>b</sup>                     |
| 7                       | CO <sub>2</sub> | 16.05 <sup>a</sup>  | 16.58 <sup>a</sup>                     | 29.95 <sup>a</sup>                     |
|                         | O <sub>2</sub>  | 4.55 <sup>a</sup>   | 1.47 <sup>b</sup>                      | 1.84 <sup>b</sup>                      |
|                         | N <sub>2</sub>  | 79.80 <sup>a</sup>  | 81.96 <sup>a</sup>                     | 69.22 <sup>b</sup>                     |
| 14                      | CO <sub>2</sub> | 19.88 <sup>a</sup>  | 17.27 <sup>a</sup>                     | 24.96 <sup>b</sup>                     |
|                         | O <sub>2</sub>  | 1.34 <sup>b</sup>   | 1.05 <sup>b</sup>                      | 3.93 <sup>a</sup>                      |
|                         | N <sub>2</sub>  | 78.79 <sup>a</sup>  | 81.67 <sup>a</sup>                     | 71.10 <sup>b</sup>                     |
| 21                      | CO <sub>2</sub> | 21.48 <sup>a</sup>  | 16.70 <sup>a</sup>                     | 22.78 <sup>b</sup>                     |
|                         | O <sub>2</sub>  | 1.09 <sup>b</sup>   | 2.56 <sup>b</sup>                      | 0.86 <sup>b</sup>                      |
|                         | N <sub>2</sub>  | 76.89 <sup>a</sup>  | 80.76 <sup>a</sup>                     | 76.34 <sup>a</sup>                     |
| 28                      | CO <sub>2</sub> | 20.41 <sup>a</sup>  | 18.54 <sup>a</sup>                     | 23.66 <sup>b</sup>                     |
|                         | O <sub>2</sub>  | 2.05 <sup>b</sup>   | 0.39 <sup>b</sup>                      | 0.51 <sup>b</sup>                      |
|                         | N <sub>2</sub>  | 77.54 <sup>a</sup>  | 82.47 <sup>a</sup>                     | 75.85 <sup>a</sup>                     |

<sup>a,b</sup>Means in the same column bearing a common superscript letter are not different ( $P > .05$ ).

percentages of CO<sub>2</sub>: (a) increased significantly between 0 and 7 days of storage but did not change thereafter in the vacuum-packages, (b) did not change during storage in the 20% CO<sub>2</sub> + 80% N<sub>2</sub> treatment and (c) decreased between 7 and 14 days of storage but not thereafter in the 40% CO<sub>2</sub> + 60% N<sub>2</sub> treatment. The relative percentages of O<sub>2</sub> decreased ( $P < .05$ ) between either 0 and 7 days (20% CO<sub>2</sub> + 80% N<sub>2</sub> and 40% CO<sub>2</sub> + 60% N<sub>2</sub> packages) or 7 and 14 days (vacuum-packages) during storage; these results are consistent with those reported by Seideman et al. (13). The relative weight percentages of N<sub>2</sub> increased ( $P < .05$ ) slightly during storage for both of the modified gas atmosphere treatments. The initial percentage of nitrogen reported for vacuum-packaged pork loins differs from that reported by Seideman et al. (13), possibly because of the difficulty in completely evacuating atmospheric air from the headspaces of packages in the present study.

Since CO<sub>2</sub> has an inhibitory effect on microorganisms normally associated with spoilage of fresh meats (5,11), it seems reasonable to package pork in modified atmospheres containing relatively high concentrations of CO<sub>2</sub>. Direct addition of CO<sub>2</sub> should be advantageous since there is a lag time associated with the increase in concentration of CO<sub>2</sub> in wholesale cuts of pork which are vacuum-packaged.

Mean values for certain evaluations of pork subprimal cuts are presented in Table 3. Packaging treatment had essentially no effect on muscle color, off-odor, fat cover appearance, muscle surface discoloration or overall appearance. As storage interval increased: (a) vacuum-packaged cuts had more ( $P < .05$ ) off-odor, (b) cuts packaged in 40% CO<sub>2</sub> + 60% N<sub>2</sub> had more ( $P < .05$ ) off-odor, and (c) cuts packaged in 20% CO<sub>2</sub> + 80% N<sub>2</sub> had less desirable ( $P < .05$ ) fat cover appearance but less extensive muscle surface discoloration. Others (2,12)

have studied vacuum-packaged beef and reported few significant differences in off-odor, fat cover appearance, muscle surface discoloration or overall appearance through 14 or 21 days of storage.

Mean surface discoloration and overall appearance ratings for retail loin chops from each packaging treatment are presented in Table 4. Few significant differences were observed as a result of packaging treatment for either surface discoloration or overall appearance regardless of storage interval or day of retail display. In three instances, retail chops from vacuum-packaged loins, had lower ( $P < .05$ ) ratings for surface discoloration or overall appearance than did cuts stored in either of the modified atmospheres. As storage interval increased, within the same day of retail display, surface discoloration generally increased and overall appearance generally decreased. During the 5-day retail display period, surface discoloration and overall appearance ratings generally decreased (within storage intervals). Since chops from vacuum-packaged loins generally had more surface discoloration and less desirable overall appearance when there were significant packaging treatment differences, modified atmospheric packaging

appears to be advantageous in assuring satisfactory appearance of subsequent retail cuts during retail display. This is especially important since some consumer studies have shown that physical appearance is the most important factor used in the selection of retail cuts by the consumer (6, 7, 8).

Mean sensory panel ratings for cooked pork chops are presented in Table 5. Chops from loins stored in 40%  $\text{CO}_2$  + 60%  $\text{N}_2$  for 7 days were more juicy ( $P < .05$ ) and chops from loins stored in 40%  $\text{CO}_2$  + 60%  $\text{N}_2$  for 28 days were less juicy ( $P < .05$ ) than were chops from loins stored in vacuum packages or in 20%  $\text{CO}_2$  + 80%  $\text{N}_2$  for comparable storage intervals. Otherwise there were no differences in palatability associated with prior packaging treatment of subprimal cuts. As storage interval increased, few significant differences were observed with regard to ratings for juiciness, flavor or overall palatability.

As was previously mentioned,  $\text{CO}_2$  has been demonstrated to have an inhibitory effect on microorganisms normally associated with the spoilage of fresh meats (14). Vacuum-packaging has been shown to extend the shelf life of fresh pork (14); this benefit is, at least partially,

TABLE 3. Means for color, off-odor, discoloration and appearance of pork subprimal cuts in each storage interval and packaging treatment.

| Trait                                     | Storage interval (days) | Packaging treatment |                   |                  |                                    |
|-------------------------------------------|-------------------------|---------------------|-------------------|------------------|------------------------------------|
|                                           |                         | Vacuum packaged     | 40% $\text{CO}_2$ | 60% $\text{N}_2$ | 20% $\text{CO}_2$ 80% $\text{N}_2$ |
| Muscle color <sup>d</sup>                 | 7                       | 6.8a                | 6.4b              | 6.4a             |                                    |
| Muscle color                              | 14                      | 6.8a                | 6.5b              | 7.3a             |                                    |
| Muscle color                              | 21                      | 7.1a                | 7.8a              | 7.0a             |                                    |
| Muscle color                              | 28                      | 7.1a                | 6.5b              | 7.0a             |                                    |
| Off-odor <sup>e</sup>                     | 7                       | 4.0a                | 4.0a              | 3.7a             |                                    |
| Off-odor                                  | 14                      | 3.4a                | 3.5bc             | 3.7a             |                                    |
| Off-odor                                  | 21                      | 3.0b                | 3.8ab             | 3.9a             |                                    |
| Off-odor                                  | 28                      | 3.0b                | 3.1c              | 3.5a             |                                    |
| Fat cover appearance <sup>f</sup>         | 7                       | 5.9a                | 5.8ab             | 6.0a             |                                    |
| Fat cover appearance                      | 14                      | 6.2a                | 6.1ab             | 6.4a             |                                    |
| Fat cover appearance                      | 21                      | 6.0a                | 6.5a              | 6.2a             |                                    |
| Fat cover appearance                      | 28                      | 5.5a                | 5.5b              | 4.6b             |                                    |
| Muscle surface discoloration <sup>g</sup> | 7                       | 5.4a                | 4.3a              | 3.3c             |                                    |
| Muscle surface discoloration              | 14                      | 4.7a                | 4.3a              | 6.1ab            |                                    |
| Muscle surface discoloration              | 21                      | 4.9a                | 4.4a              | 4.9b             |                                    |
| Muscle surface discoloration              | 28                      | 4.4a                | 4.3a              | 6.3a             |                                    |
| Overall appearance <sup>h</sup>           | 7                       | 6.1a                | 5.3a              | 4.3a             |                                    |
| Overall appearance                        | 14                      | 5.6a                | 5.4a              | 6.2a             |                                    |
| Overall appearance                        | 21                      | 4.8a                | 5.0a              | 5.3a             |                                    |
| Overall appearance                        | 28                      | 5.0a                | 4.8a              | 5.5a             |                                    |

<sup>abc</sup>Means in the same column for the same trait bearing a common superscript letter do not differ ( $P > .05$ ). Mean values within the same day of storage for each trait which are underscored by a common line do not differ ( $P > .05$ ).

<sup>d</sup>Means based on a 9-point scale (9 = very light cherry red; 1 = very dark purple).

<sup>e</sup>Means based on a 4-point scale (4 = no detectable off-odor; 1 = extreme off-odor).

<sup>fg</sup>Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

<sup>h</sup>Means based on an 8-point scale (8 = extremely desirable, 1 = extremely undesirable).

due to the accumulation of CO<sub>2</sub> in the vacuum package, thus there is a lag time required after vacuum-packaging to allow buildup of CO<sub>2</sub>. Results of the present study indicate that advantage can be taken of the desirable microbial inhibitory effect of CO<sub>2</sub> with no apparent disadvantages regarding appearance of the primal cuts, caselife of retail cuts or sensory properties of cooked cuts even after extended periods of storage. Although these

results are encouraging, further research is warranted to identify the optimum gas combination for modified atmosphere storage of fresh pork.

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TABLE 4. Means for surface discoloration and overall appearance for retail loin chops stratified according to day of retail display, storage interval and packaging treatment.

| Day of retail display | Storage interval (days) | Surface discoloration <sup>e</sup> |                                        |                                        | Overall appearance <sup>f</sup> |                                        |                                        |
|-----------------------|-------------------------|------------------------------------|----------------------------------------|----------------------------------------|---------------------------------|----------------------------------------|----------------------------------------|
|                       |                         | Vacuum packaged                    | 40% CO <sub>2</sub> 60% N <sub>2</sub> | 20% CO <sub>2</sub> 80% N <sub>2</sub> | Vacuum packaged                 | 40% CO <sub>2</sub> 60% N <sub>2</sub> | 20% CO <sub>2</sub> 80% N <sub>2</sub> |
| 1                     | 0                       | 6.5a                               | 6.5a                                   | 6.5a                                   | 6.9a                            | 6.9a                                   | 6.9a                                   |
|                       | 7                       | 6.1ab                              | 6.3a                                   | 6.2a                                   | 6.4ab                           | 6.7a                                   | 6.4a                                   |
|                       | 14                      | 5.8ab                              | 5.9ab                                  | 5.9a                                   | 6.6ab                           | 6.3a                                   | 6.7a                                   |
|                       | 21                      | 4.5c                               | 5.8ab                                  | 5.8a                                   | 4.5c                            | 5.6a                                   | 5.7a                                   |
|                       | 28                      | 5.4b                               | 4.9b                                   | 5.9a                                   | 5.6bc                           | 5.4a                                   | 6.3a                                   |
| 3                     | 0                       | 6.4a                               | 6.4a                                   | 6.4a                                   | 6.6a                            | 6.6a                                   | 6.6a                                   |
|                       | 7                       | 4.9b                               | 5.0a                                   | 3.9c                                   | 5.5a                            | 5.4ab                                  | 3.7c                                   |
|                       | 14                      | 4.9b                               | 4.7a                                   | 5.2ab                                  | 5.5a                            | 5.1ab                                  | 5.2b                                   |
|                       | 21                      | 3.9b                               | 5.4a                                   | 4.9bc                                  | 3.9b                            | 5.4ab                                  | 5.2b                                   |
|                       | 28                      | 3.8b                               | 4.6a                                   | 4.9bc                                  | 3.5b                            | 4.7b                                   | 4.6bc                                  |
| 5                     | 0                       | 5.0a                               | 5.0a                                   | 5.0a                                   | 5.2a                            | 5.2a                                   | 5.2a                                   |
|                       | 7                       | 3.9ab                              | 4.9a                                   | 3.0b                                   | 4.4ab                           | 4.2bc                                  | 2.7d                                   |
|                       | 14                      | 4.4a                               | 4.8a                                   | 4.6a                                   | 4.4ab                           | 4.3ab                                  | 4.4b                                   |
|                       | 21                      | 2.4bc                              | 4.1ab                                  | 3.9ab                                  | 2.6b                            | 3.7bc                                  | 3.6c                                   |
|                       | 28                      | 2.3c                               | 2.7b                                   | 2.5b                                   | 2.8b                            | 2.8c                                   | 2.6d                                   |

abcd Means in the same column for the same day of retail display bearing a common superscript letter are not different ( $P > .05$ ). Means within the same day of storage for each trait which are underscored by a common line are not different ( $P > .05$ ).

<sup>e</sup>Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

<sup>f</sup>Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

TABLE 5. Mean sensory panel ratings for cooked pork chops stratified according to storage interval and packaging treatment.

| Palatability Rating               | Storage interval (days) | Packaging treatment |                                        |                                        |
|-----------------------------------|-------------------------|---------------------|----------------------------------------|----------------------------------------|
|                                   |                         | Vacuum packaged     | 20% CO <sub>2</sub> 80% N <sub>2</sub> | 40% CO <sub>2</sub> 60% N <sub>2</sub> |
| Juiciness <sup>c</sup>            | 0                       | 4.4a                | 4.4a                                   | 4.4a                                   |
| Juiciness                         | 7                       | 4.3a                | 4.1a                                   | 4.7a                                   |
| Juiciness                         | 14                      | 3.9ab               | 3.4b                                   | 4.0a                                   |
| Juiciness                         | 21                      | 3.4b                | 3.9ab                                  | 3.4ab                                  |
| Juiciness                         | 28                      | 4.4a                | 4.2a                                   | 3.0b                                   |
| Flavor desirability <sup>d</sup>  | 0                       | 4.9a                | 4.9a                                   | 4.9a                                   |
| Flavor desirability               | 7                       | 4.8a                | 5.0a                                   | 5.4a                                   |
| Flavor desirability               | 14                      | 4.5a                | 4.9a                                   | 5.0a                                   |
| Flavor desirability               | 21                      | 4.5a                | 5.2a                                   | 4.8a                                   |
| Flavor desirability               | 28                      | 5.1a                | 4.9a                                   | 4.6a                                   |
| Overall palatability <sup>e</sup> | 0                       | 4.9a                | 4.9a                                   | 4.9a                                   |
| Overall palatability              | 7                       | 4.8a                | 4.9a                                   | 5.3a                                   |
| Overall palatability              | 14                      | 4.4a                | 4.6a                                   | 4.8a                                   |
| Overall palatability              | 21                      | 3.9b                | 5.0a                                   | 4.5a                                   |
| Overall palatability              | 28                      | 4.9a                | 4.8a                                   | 4.3a                                   |

ab Means in the same column and for the same palatability trait bearing a common superscript letter are not different ( $P > .05$ ). Means within the same day of storage and for the same trait which are underscored by a common line are not different ( $P > .05$ ).

<sup>c</sup>Means based on an 8-point scale (8 = extremely juicy; 1 = extremely dry).

<sup>d</sup>e Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

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## Effect of Retail Sanitation on the Bacterial Load and Shelf Life of Beef

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### ABSTRACT

The influence of retail sanitation on the psychrotrophic bacterial load and subsequent shelf life of rib steaks was investigated under laboratory-simulated retail processing conditions. Steaks were fabricated employing extensively sanitized processing equipment ( $< 10$  bacteria/cm<sup>2</sup> of surface) or with highly contaminated equipment where bacterial counts approached  $10^6$ /cm<sup>2</sup>. No significant differences were observed in the initial psychrotrophic bacterial load, bacterial growth rate or in the organoleptic deterioration of steaks processed under these extremes of retail sanitation. These results were confirmed with ground beef fabricated under similar extremes of grinding sanitation. Although the psychrotrophic bacterial load on steaks could not be related to the degree of retail processing sanitation, it was significantly correlated with the level of surface contamination on wholesale ribs. In addition, the extent of steak surface discoloration and overall retail appearance were significantly correlated to the psychrotrophic bacterial load.

The microbial quality of fresh meat is dependent upon the microbial contamination on the live animal and a multiplicity of processing factors from slaughter to retail display. At the abattoir, the major sources of carcass contamination are the hides, hooves and gut contents of slaughter animals (1,2). Subsequent dressing procedures and retail processing and packaging may contribute to the microbial load through increased contact with contaminated processing equipment, poor personnel hygiene and improper temperature control (1,2,3,5,11,17). Relative to this, many investigators have stressed the importance of the degree of retail sanitation to the microbial quality and safety of meats (1,2,3,5,19). Conversely, some workers have contended that the bacterial contamination of ground beef is more dependent upon the quality of the meat utilized in fabrication than on the conditions of sanitation during grinding (4,7,16,20). These investigators concluded that the bacterial load of beef patties represented a cumulative contamination from slaughter to the packaging of trimmings and contaminated retail equipment surfaces contributed little to the ultimate microbial quality of the patties.

In general, there is a lack of evidence clearly correlating the bacterial population of contaminated retail processing equipment and personnel to the bacterial load and subsequent spoilage of retail cuts. In view of this, the present study was designed and conducted to more fully document the importance of retail sanitation to the microbial quality and retail shelf

life of beef cuts by simulating different levels of retail sanitation in the laboratory.

### MATERIALS AND METHODS

#### Steak processing conditions

Wholesale beef ribs were obtained from cattle slaughtered at a Government inspected abattoir. After six days of aging in the laboratory (2 C, 90% RH), rib steaks were fabricated under three levels of sanitation: (a) steaks were cut following sterilization of the saw, knives, cutting table and meat handlers' apparel (where autoclaving was not practical, surfaces were sanitized with 5% sodium hypochlorite and rinsed with 70% ethanol immediately before use); (b) after cutting the first series of steaks, the equipment and apparel were maintained at room temperature ( $22.5 \pm 1.5$  C) for 24 h without cleaning, and a second series of steaks was cut, and (c) the equipment and apparel were left without cleaning for a total of 72 h before cutting the last series of steaks. Under each sanitation level, a total of 35 rib steaks was cut and wrapped in an oxygen-permeable polyvinyl film (vitafilm choice wrap) and displayed in a self service display case (Hill Refrigeration of Canada Ltd.) at 2 C under incandescent lighting (82 fc).

#### Steak sensory evaluation and bacterial sampling

At daily intervals, from day 0 to day 7, five randomly selected steaks were evaluated by a five member sensory panel for the extent of surface discoloration and for consumer acceptability using the scale presented in Table 1. In addition, the same samples were swabbed to determine the psychrotrophic bacterial load.

Microbiological sampling was conducted by swabbing an area of 10 cm<sup>2</sup> (defined by a sterile aluminum template), using a sterile swab moistened in a 0.1% peptone-water (14). Immediately before cutting, five wholesale ribs were sampled by swabbing four locations on each including the external subcutaneous fat (2 swabs), the anterior and posterior ends and the interior intercostal muscles. A total surface area of 50 cm<sup>2</sup> was sampled and the swabs were pooled. The meat contact surfaces were sampled as follows: saw, 5 × 10 cm<sup>2</sup> swabs; steak scraping

TABLE 1. *Steak sensory evaluation scales.*

#### Discoloration scale

1. No surface discoloration
2. 1-10% surface discoloration
3. 11-25% surface discoloration
4. 26-50% surface discoloration
5. 51-75% surface discoloration
6. 76-100% surface discoloration
7. Brown
8. Green/grey

#### Retail appearance scale

1. Extremely undesirable
2. Unsaleable
3. Slightly undesirable
4. Neither desirable or undesirable
5. Slightly desirable
6. Desirable
7. Extremely desirable

knife,  $1 \times 10$  cm<sup>2</sup> swab, and the cutting table,  $2 \times 10$  cm<sup>2</sup> swabs. The meat cutter's apparel was sampled by swabbing the gloves ( $2 \times 10$  cm<sup>2</sup> swabs) and apron ( $1 \times 10$  cm<sup>2</sup> swab). After swabbing, bacterial samples were diluted in 0.1% peptone-water and psychrotrophic bacteria were enumerated following the surface plating of 0.1 ml of the appropriate dilutions on Plate Count Agar (Difco) and incubation at 4 C for 7 days.

#### Ground beef processing conditions and bacterial sampling

In a complementary study, the short ribs removed from the wholesale ribs under conditions 1, 2 and 3 (previously described) were used for fabrication of ground beef. Thus, short ribs obtained from wholesale ribs employing highly sanitized equipment (condition 1) were processed under highly sanitary grinding conditions. Similarly, short ribs derived under septic conditions (2 and 3) were processed using corresponding levels of sanitation during grinding. The meat contact surfaces were sampled prior to each day's processing as follows: boning knife,  $1 \times 10$  cm<sup>2</sup> swab; cutting table,  $2 \times 10$  cm<sup>2</sup> swabs, and grinder,  $5 \times 10$  cm<sup>2</sup> swabs and  $1 \times 4$  cm<sup>2</sup> swab. The boner's and grinder's gloves ( $2 \times 10$  cm<sup>2</sup> swabs) and aprons ( $1 \times 10$  cm<sup>2</sup> swab) were also sampled. Immediately before each day's processing, 10 cm<sup>2</sup> of surface area of each of five randomly selected short ribs were swabbed to determine the initial bacterial load.

For each of the three levels of grinding sanitation, 25 patties weighing approximately 250 g were formed, wrapped in oxygen-permeable film and displayed in the retail display case. Five patties were removed from retail display at daily intervals and swabbed ( $10$  cm<sup>2</sup>) to determine the psychrotrophic bacterial load as previously described. No sensory evaluation of these samples was conducted.

#### Statistical analyses

Significant differences between bacterial growth rates were determined by comparing the slopes of the logarithmic phase of the growth curves, using the Student's 't' test (13). In all other instances, the treatment means were compared by analysis of variance followed by Duncan's Multiple Range test (13).

## RESULTS

### Level of equipment sanitation in processing steaks and ground beef

The psychrotrophic bacterial loads on meat contact surfaces exposed to different levels of sanitation during steak and ground beef processing are shown in Fig. 1 and 2, respectively. In both instances, bacterial contamination was negligible following extensive sanitation (log bacteria/cm<sup>2</sup> ranged from 0.30 to 1.00) but increased significantly ( $p < 0.001$ ) at all locations sampled (log bacteria/cm<sup>2</sup> ranged from 4.20 to 6.05) when surfaces were not cleaned for 24 and 72 h after beef processing. It is noteworthy that on the majority of surfaces sampled there was a slight decrease in psychrotrophic bacterial contamination at 72 h when compared to 24 h. This would be expected providing conditions were such that the organisms had entered the late stationary or early death phase of bacterial growth. Alternatively, psychrotrophic growth may have been suppressed by antagonistic mesophilic proliferation.

### Bacterial growth on steaks and ground beef

Rib steaks and ground beef were fabricated using the three levels of retail processing sanitation previously described and the psychrotrophic bacterial growth on sample surfaces is shown in Fig. 3 and 4, respectively. No statistically significant differences were found in the initial psychrotrophic bacterial load ( $p > 0.01$ ) or in the rates of bacterial growth ( $p > 0.10$ ) on samples prepared

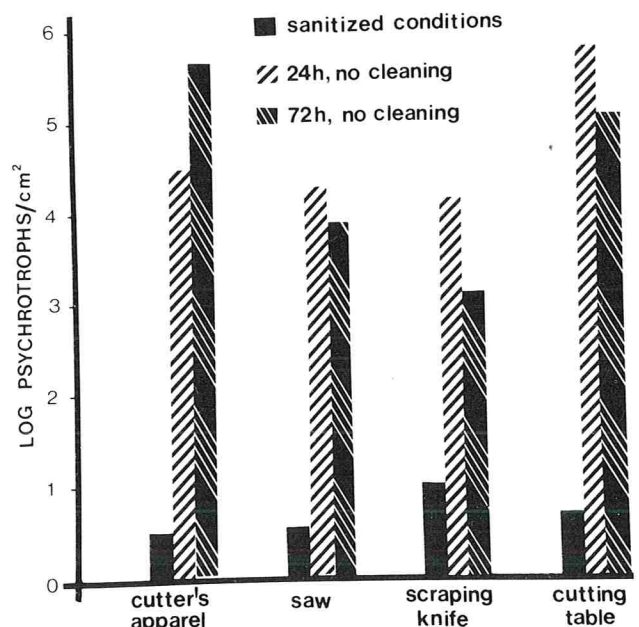


Figure 1. Effects of the degree of retail sanitation on the psychrotrophic bacterial load on meat contact surfaces employed for steak processing.

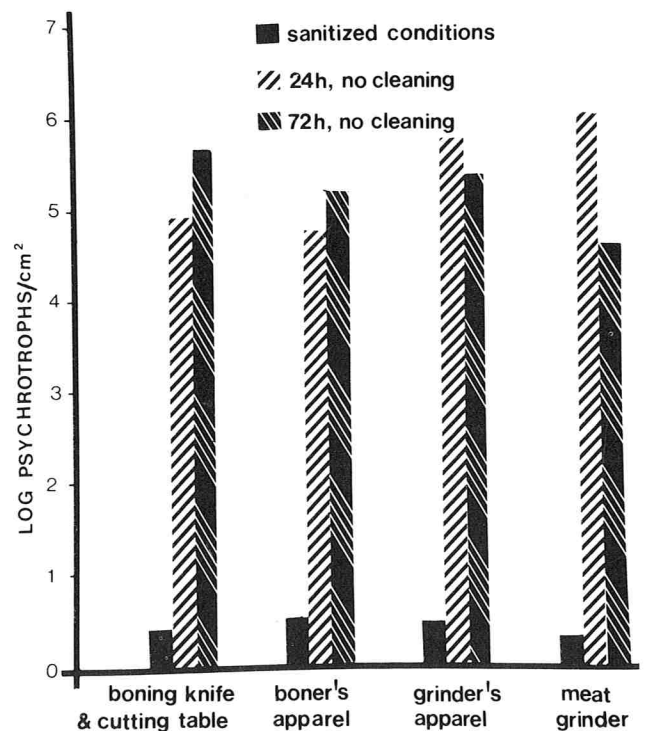


Figure 2. Effects of the degree of retail sanitation on the psychrotrophic bacterial load on meat contact surfaces employed for ground beef processing.

under the three levels of sanitation. Moreover, there was no significant correlation ( $p > 0.05$ ) between the degree of processing sanitation and the initial psychrotrophic bacterial load on steaks or ground beef patties.

### Sensory evaluation of steaks

Steaks fabricated under the three levels of retail sanitation were also evaluated for surface discoloration (Fig. 5) and for retail appearance (Fig. 6). In all



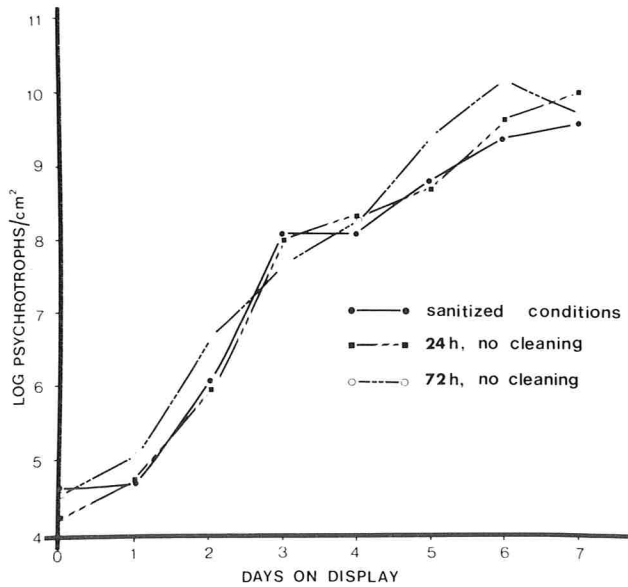


Figure 3. Effects of the degree of processing sanitation on the initial psychrotrophic bacterial load and bacterial growth on steaks.

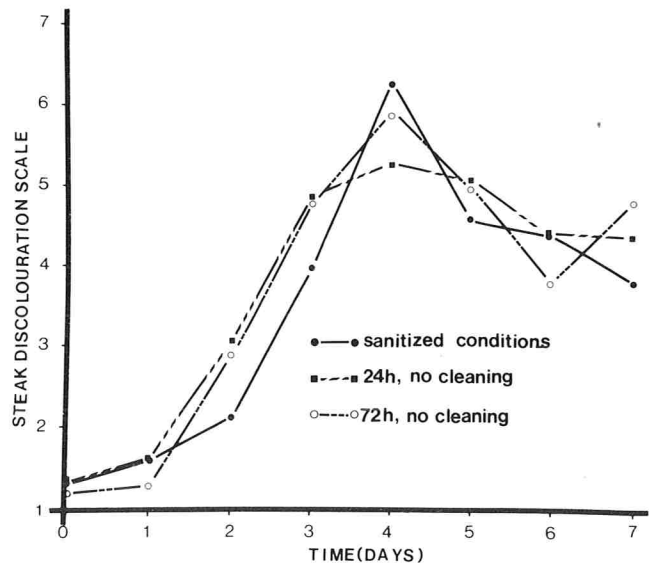


Figure 5. Effects of the degree of processing sanitation on steak surface discoloration. Steak surface discoloration was evaluated subjectively by means of an 8 point scale (1 = no discoloration; 8 = green or grey).

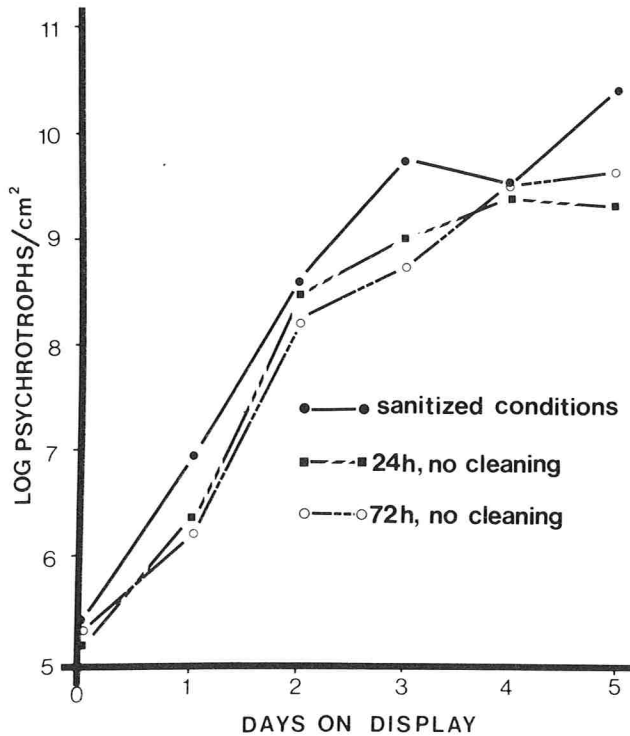


Figure 4. Effects of the degree of processing sanitation on the initial psychrotrophic bacterial load and bacterial growth on ground beef.

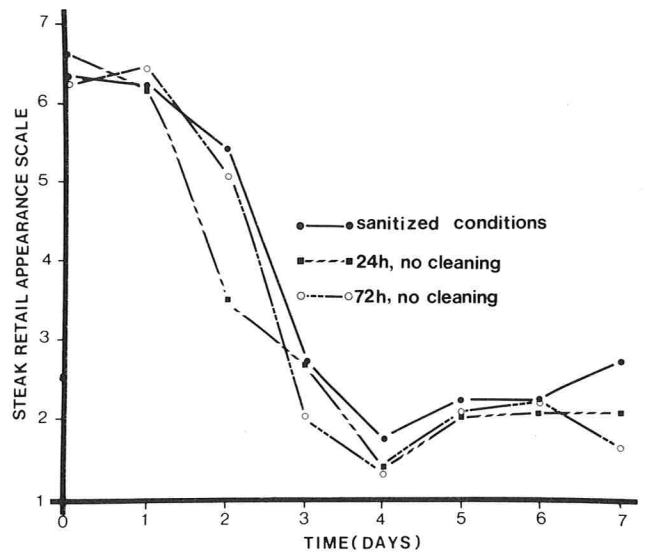


Figure 6. Effects of the degree of processing sanitation on steak retail appearance. Steak retail appearance was evaluated by means of a 7 point hedonic scale (1 = extremely undesirable; 7 = extremely desirable).

instances, there was a progressive increase in the amount of surface discoloration (Fig. 5) and a progressive decrease in overall appearance (Fig. 6) with time on retail display. However, no significant differences in either discoloration ( $P > 0.10$ ) or retail appearance ( $P > 0.10$ ) were observed when the processing sanitation levels were compared.

Analyses of data with sanitation levels pooled by linear regression and correlation revealed that steak surface discoloration (Fig. 7;  $r = 0.870$ ,  $P < 0.01$ ) and retail

appearance (Fig. 8,  $r = 0.921$ ,  $P < 0.01$ ) were significantly related to the psychrotrophic bacterial load on steak surfaces. In view of this and the similarity of bacterial counts reported in Fig. 3, it is not surprising that the deterioration in visual acceptability of steaks cut employing contaminated equipment was similar to that of samples cut using sanitized equipment.

*Effects of wholesale rib contamination on steak bacterial loads*

Throughout this and other investigations in our laboratory, a large variation in the psychrotrophic bacterial load on wholesale ribs was noted (log bacteria/cm<sup>2</sup> from 2.11 to 7.15). The data presented in Fig. 9

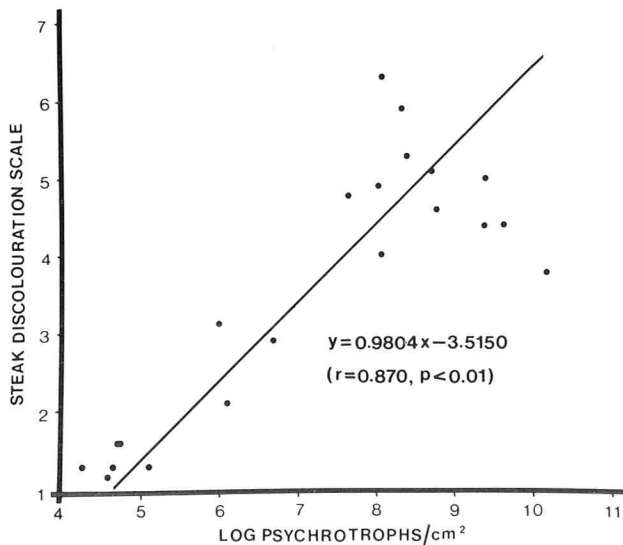


Figure 7. Relationship between steak psychrotrophic bacterial loads and discoloration.

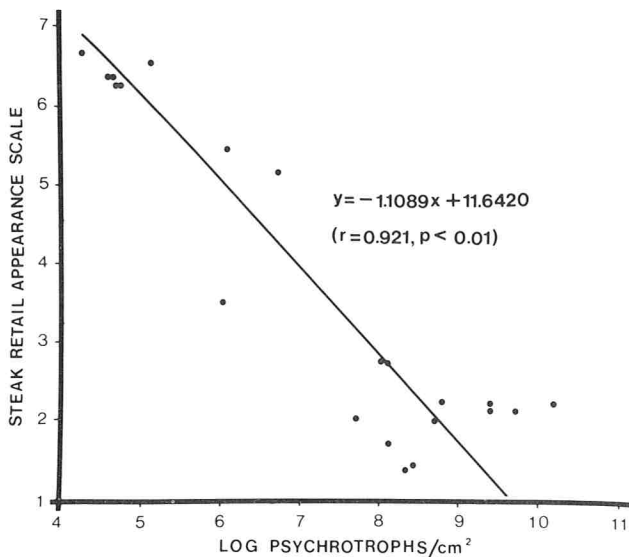


Figure 8. Relationship between steak psychrotrophic bacterial loads and retail appearance.

shows a direct correlation between the bacterial contamination on ribs and the initial bacterial load on the surface of corresponding rib steaks, fabricated using sanitary processing procedures ( $r = 0.879$ ,  $P < 0.01$ ).

In the present study, the log bacterial counts / $\text{cm}^2$  on the surface of wholesale ribs, immediately before the fabrication of steaks, were as follows: 6.16 for ribs to be processed under sanitary conditions and 7.20 and 7.28 for ribs to be processed after 24 and 72 h with no equipment sanitation, respectively. Although the contamination on ribs employed in processing steaks with sanitized equipment was significantly lower ( $P < 0.05$ ) when compared to the others, this difference was not large enough to result in any significant differences in the initial bacterial loads on steak surfaces (Fig. 3).

It is of importance to note that the bacterial counts on short ribs, utilized for ground beef fabrication, were not

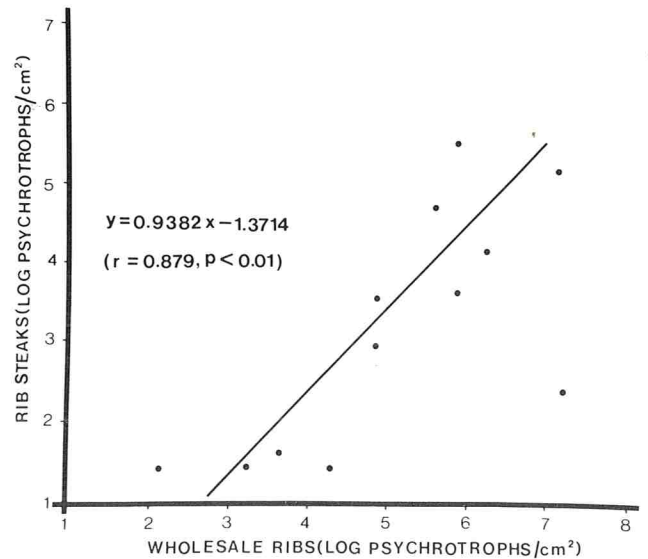


Figure 9. Relationship between the psychrotrophic bacterial load on wholesale ribs and the initial load on steaks.

significantly different ( $p > 0.10$ ; log bacteria/ $\text{cm}^2$  was 6.59 for short ribs to be processed with sanitized equipment and 5.62 and 5.76 for short ribs processed after 24 and 72 h of no equipment sanitation, respectively). No attempt was made to correlate short rib bacterial loads with those observed on ground beef patties.

## DISCUSSION

The unexposed muscle tissues of healthy animals are considered to be virtually sterile (8), particularly with regard to psychrotrophic spoilage bacteria (18). Consequently, the contamination on retail cuts results from the contaminated surfaces of carcasses or primal cuts (2,9,10,15,16,20) and/or unsanitary retail processing conditions (1,3,5,15,19,20). The latter possibility was investigated in the current study by fabricating retail beef cuts using extensively sanitized processing equipment ( $< 10$  bacteria/ $\text{cm}^2$  of surface) or with highly contaminated equipment where bacterial counts approached  $10^6/\text{cm}^2$ . Unexpectedly, no significant differences were observed in the initial psychrotrophic bacterial load, bacterial growth rate or in the visual deterioration of rib steaks processed under these extremes of simulated retail sanitation. These findings were further substantiated with ground beef fabricated under similar extremes of grinding sanitation.

The present observations are contrary to the contentions of several other workers who have reported that bacterial loads on retail processing equipment contribute to the ultimate bacterial quality of retail cuts (3,5,19,20). There is, however, a lack of definitive data attempting to differentiate the relative contribution of unsanitary retail processing and contaminated carcass or wholesale cuts to the bacterial load on retail cuts. Relative to this, Bronsdon (3) showed a pronounced reduction in bacterial counts and growth rate and an extended case life for meats as a result of a rigorous

sanitation program in a retail store. However, no data were given for the level of contamination on the wholesale product employed for the fabrication of retail cuts, nor were any processing equipment counts reported. Additional studies by West et al. (20) led to the conclusion that the majority of psychrotrophic bacterial contamination on steaks resulted from use of unsanitary retail equipment as opposed to that derived from the external surfaces of the primal cuts. No attempt, however, was made to correlate equipment or primal cut bacterial loads with the extent of steak contamination. In direct contrast, the results of the present study clearly showed a significant positive correlation between the surface contamination of wholesale ribs and the initial psychrotrophic bacterial load on corresponding rib steaks. Furthermore, the extent of steak surface discoloration and subsequent visual desirability of rib steaks were directly related to the psychrotrophic bacterial load. In view of this and the similarity of bacterial counts on rib surfaces, the spoilage pattern during retail display was identical, irrespective of the level of processing sanitation.

It would appear, from the present results, that the level of wholesale rib surface contamination had reached the extent where highly unsanitary processing procedures contributed little to the ultimate bacterial load on retail rib steaks. If this was the case, it would be of value to establish a level of rib contamination where the degree of retail sanitation did have an effect. Presumably, at very low rib bacterial loads the degree of retail sanitation would become of more importance to the microbial quality of retail cuts.

One aspect of the present study is in general agreement with the findings of other (4,7,16) in that the degree of psychrotrophic bacterial contamination of ground beef was not related to the level of sanitation employed during grinding. In this regard, although West and co-workers (20) stressed the importance of retail sanitation to the eventual shelf life of steaks, they noted that the relatively high bacterial loads in ground beef arose from contaminated raw beef in contrast to unsanitary processing. More recently, Surkiewicz et al. (16) showed a positive association between the aerobic plate counts of beef trimmings and that of fabricated patties. The results of the present study have extended these observations by demonstrating that the microbial quality of rib steaks is also related to the bacterial load on the wholesale beef employed for processing.

In view of the increasing trend towards fabricating ground beef, primal and subprimal cuts at centralized packers (12) a concomitant decrease in retail processing has resulted. As a consequence, it has become important to establish to what extent initial bacterial loads on carcasses and the increased handling during centralized processing influences the ultimate bacterial quality and shelf life of retail cuts. Since Emswiler et al. (6) reported that the retail case life of beef was inversely related to the level of carcass contamination, the highly variable

bacterial load on carcass surfaces (9,10) becomes an important consideration. Relative to this, in an extensive study of the bacterial quality of beef carcasses, Stringer and co-workers (15) showed a progressive increase in beef contamination following slaughter, dressing and carcass cooling with a pronounced increase occurring during transport to the retail store. Complementary studies by other researchers (20) indicated that the bacterial counts on rib and loin surfaces increased by more than 100-fold during fabrication from beef quarters at a beef distribution center. In consideration of these findings and the results of the present study, it is not unlikely that even in retail outlets employing strictly hygienic processing procedures, spoilage losses and inability to meet proposed bacteriological standards (21) may result as a consequence of highly contaminated carcasses and/or wholesale cuts.

#### ACKNOWLEDGMENTS

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## The Most Suitable Number of Colonies on Plates for Counting<sup>1</sup>

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### ABSTRACT

Major events that led to acceptance of 30 to 300 as the most suitable number of colonies on plates for counting were reviewed. Three new sets of data were collected, involving triplicate plates of fifteen 1:1.4 serial dilutions of 65 samples of raw milk. Statistical methods were developed to analyze bias (variability introduced primarily by crowding and analyst counting errors) and variance (sampling and dilution errors). Bias and variance were combined as mean-squared error, which was expressed as a function of the number of colonies per plate. The counting range that minimized the mean squared error could then be determined for selected dilution series. For two-fold, five-fold and ten-fold dilution series, respectively, the most suitable limits on plates for counting were 70 to 140, 40 to 200 and 25 to 250 colonies/plate. A range of 25 to 250 colonies/plate was suggested for the analysis of dairy products. Limitations in application of the data to other systems are discussed.

Almost every textbook, laboratory manual and methods volume in microbiology contains the statement that plates for counting bacteria should contain, when possible, 30-300 colonies. The "30-300" concept has been so ingrained in our thinking that the limits are rarely questioned. We report here the historical background of the "30-300" concept and results of a study wherein modern plating methods were analyzed statistically to determine the counting range that would result in minimal errors when making plate counts.

The "30-300" concept originated with two publications by Breed and Dotterer (7,8); the text and tables presented in both publications are identical. The authors summarized results of a few early studies and then proceeded to more clearly define the problem and provide a solution. Three dilutions ( $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ ) of each of a large number of raw milk samples were plated in triplicate on standard agar. Colonies were counted after incubation for 5 days at 20 C and again after incubation for 2 additional days at 37 C. Colonies on all plates were counted, when possible. Individual plate

counts were tabulated as "satisfactory" if the count could be used in making an average without deviating by more than 20% from the average. Thus, each average was determined by a variable number of plates in the serial dilution series for a sample; occasionally, all nine of the plates made from a sample could be included in the final average. An individual plate count was tabulated as a "discrepancy" if the count varied by more than 20% from the average.

The 21 C, 5-day data of Breed and Dotterer (7,8) are reproduced in Table 1 because these data have, through the years, served as the major basis for specifying the most suitable number of colonies on plates for counting. Their description of the data (slightly paraphrased) follows:

"Table 1 gives the number of plate counts made after 5 days at 21 C, arranged in groups according to the number of colonies which appeared on the plates. Of the 1435 plates, 439 had less than 10 colonies/plate. Only 22% of these checked within the 20% limit. Of 180 plates in the group having more than 10 and less than 20 colonies/plate, 54% checked within the 20% limit. Percentages calculated for the other groups of plates were more or less variable, showing that from 66 to 93% of the total number of plates agreed within the 20% limit. The best percentage of agreement is shown by the group having more than 100 and less than 200 colonies/plate, and the next highest by the group having between 50 and 100 colonies/plate. There were decidedly fewer plates giving satisfactory results among those which had more than 400 colonies/plate, the percentage of plates which checked within 20% being 44.

"The results given in the lower part of Table 1 were calculated from the same counts, the groups of plates having been arranged differently. From this part of the table it will be seen that the percentages of discrepant plates is practically the same for groups of plates having 20 to 400, 30 to 400, 20 to 200, 30 to 200, or 40 to 200 colonies/plate, the best showing being made by the group of plates having more than 40 and less than 200 colonies/plate. Plates having less than 30 or more than 400 colonies show very large percentages of discrepancies."

Breed and Dotterer (7,8) concluded that, for milk analyses, the counts made from plates having between

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30-400 colonies were "very nearly as satisfactory" as those obtained from plates having between 40-200 colonies. On the basis of these studies, a range of from 30-300 colonies/plate was specified for dairy products (1), and a range of from 25-250 colonies/plate for water (5). Later, the range for water was changed to 30-300 colonies/plate (6), still based on the work of Breed and Dotterrer (7). For dairy products, the bulletin by Breed and Dotterrer (8) and a paper by Hill (20) originally were cited to justify a range of from 30-300 colonies/plate. Hill (20) recommended a range of from 40-200 colonies/plate. His recommendations were based on astute observations and not statistical analyses. The reference to Hill (20) was dropped from *Standard Methods* in 1960 (2), and reference was made to the work of Wilson et al. (29) to substantiate a countable range of 30-300 colonies/plate.

Wilson et al. (29) conducted an extensive study of the magnitude of the error according to the number of colonies/plate. Samples were examined in which 2 or 3 plate counts had been made from the same dilution of milk. In every subsample, each individual value was expressed as a percentage of the mean of the subsamples. The percentages were then grouped according to the mean colony counts, and the standard deviations and coefficients of variation were calculated by using all the percentages within the groups. Wilson et al. (29) concluded that "plates with between 30-300 colonies are probably the best to count as a routine, but there is no reason why plates with up to 500 colonies should not be counted, if others are not available." How these workers arrived at a lower limit of 30 colonies is not obvious from examination of their data. The upper limit of 300 was based on observations that

"Plates with over 500 colonies under-estimate the true count owing to the overcrowding error. With careful workers the actual error of counting probably does not become appreciable till there are about 300 colonies per plate, and for some distance above this limit it will probably be counterbalanced by the diminished sampling error. If many plates, however, have to be counted, the fatigue error, which seems to

be mainly responsible for the failure of the sampling error to decrease with increasing numbers of colonies in accordance with theoretical expectations, becomes appreciable."

These "errors of counting" were analyzed by Fisher et al. (16). The difference between counts on duplicate plates was calculated for an extensive set of data on milk samples (Series C, ref. 9). Counting errors were about four times greater when over 300 colonies appeared on a plate than when lesser numbers of colonies were counted. Most of these data were obtained by adding pure cultures of *Escherichia coli* to milk; counting errors might have been greater if a mixed, natural bacterial flora had been enumerated (16).

In the present study, we attempted to combine estimates of the magnitude of error (variance) according to the number of colonies per plate (7,8,29) with estimates of errors (bias) introduced by overcrowding (7,8,26) or by counting (12,16). Statistical techniques for combining these two sources of error resulted in the mean squared error, which was expressed as a function of the number of colonies per plate. This algebraic function then was used to determine the counting range for various dilutions that would give the minimum error in determining the level of bacteria in fluid milk products.

#### MATERIALS AND METHODS

The data examined came from bacterial colony counts made on samples of raw milk obtained in three different experiments. Two experiments of 20 samples each were conducted by the U.S. Department of Health, Education and Welfare, and the data were supplied by Dr. R. B. Read, Jr.; a third experiment of 25 samples was conducted at Iowa State University by Dr. G. W. Reinbold. Preliminary standard plate counts (3) were made in duplicate, and the plates were incubated for 24 h at 32 C; the samples were held at 4 C. After the preliminary counts had been determined, fifteen 1:1.4 serial dilutions were made by using phosphate buffered water (3), and approximately 12 ml of Standard Methods agar were added to each of triplicate plates. Colonies on the plates were counted after 48-h incubation at 32 C (3). The average count of the 3 plates for each dilution was used as an estimate of the number of viable bacteria per dilution of a particular sample and was defined as the *diluted count*. Attempts were made to

TABLE 1. Plate counts after incubation at 21C arranged to show the number and percentage of counts in groups according to the number of colonies per plate (from ref. 7).

| Group      | Plates with satisfactory counts |      | Plates with discrepant counts |          |           |      | Total No. of plates in group |
|------------|---------------------------------|------|-------------------------------|----------|-----------|------|------------------------------|
|            | No.                             | %    | Too low                       | Too high | Total No. | %    |                              |
| 0 to 10    | 98                              | 22.3 | 172                           | 169      | 341       | 77.7 | 439                          |
| 10 to 20   | 97                              | 53.9 | 29                            | 54       | 83        | 46.1 | 180                          |
| 20 to 30   | 54                              | 72.9 | 6                             | 14       | 20        | 27.1 | 74                           |
| 30 to 50   | 67                              | 66.3 | 11                            | 23       | 34        | 33.7 | 101                          |
| 50 to 100  | 162                             | 84.8 | 17                            | 12       | 29        | 15.2 | 191                          |
| 100 to 200 | 179                             | 93.2 | 8                             | 5        | 13        | 6.8  | 192                          |
| 200 to 400 | 105                             | 78.9 | 25                            | 3        | 28        | 21.1 | 133                          |
| Over 400   | 100                             | 44.4 | 114                           | 11       | 125       | 55.6 | 225                          |
| 0 to 30    | 249                             | 35.9 | 207                           | 237      | 444       | 64.1 | 693                          |
| 20 to 400  | 567                             | 82.0 | 67                            | 57       | 124       | 18.0 | 691                          |
| 30 to 400  | 513                             | 83.1 | 61                            | 43       | 104       | 16.9 | 617                          |
| 20 to 200  | 470                             | 82.9 | 43                            | 54       | 97        | 17.1 | 567                          |
| 30 to 200  | 416                             | 84.3 | 37                            | 40       | 77        | 15.7 | 493                          |
| 40 to 200  | 376                             | 86.0 | 23                            | 28       | 61        | 14.0 | 437                          |
| Over 400   | 100                             | 44.4 | 114                           | 11       | 125       | 55.6 | 225                          |

obtain a diluted count of 600/ml at the lowest dilution; however, stock dilutions from which all subsequent dilutions were prepared contained from 230 to 3000 viable cells/ml. Table 2 shows diluted-count raw data for six examples taken at random from the 65 samples that were subjected to the 15 dilutions (27).

The estimated *true count*/ml (based upon a composite quadratic model applied to each of the 65 samples) was subtracted from each of the 15 diluted counts/ml to obtain a set of 15 *bias points*. Contrary to the work of Breed and Dotterer (7,8), all the points were used in the final determination of the true number of colonies/plate. A predicted bias curve was then fitted to the 975 bias points by using grafted polynomials.

Whereas the bias measured variability introduced primarily by colony crowding and analyst counting errors, the *variance* was used to measure variability due to sampling and dilution errors. To estimate the variance, a quantity referred to as *bias error* measured the difference between each bias point and the predicted bias curve. A series of grafted polynomials was fitted to the weighted squared bias error values to predict the variance of the bias points throughout the range of the sample counts/plate. The bias and the weighted estimate of the variance were combined to determine new estimates of the mean squared errors. Detailed descriptions of the statistical methods used and their development are available (27).

### RESULTS

The mean-squared-error function used to establish the optimum counting range is shown in Fig. 1. This curve is a sum of the variance function and the square of the bias for the respective values of count/plate:

$$\text{Mean squared error} = \text{Variance} + (\text{Bias})^2$$

Consequently, the mean squared error essentially is a function of the variance until the bias, due to overcrowding and technician fatigue, contributes to the error beyond 80 counts/plate. Table 3 gives values for the mean squared error for selected count/plate values. These data indicate a minimum variability in counts/ml of milk if the count is performed on plates that have about 110 counts/plate. In such an instance, the estimated count will be  $600 \pm 94$ , where 600 is the mean response and 94 is the calculated standard deviation. When counts are made on plates that range between 60 and 160 colonies/plate, the standard deviation is less than 140, whereas the standard deviation is expected to be less than 200 if the colony count/plate ranges from 25 to 250.

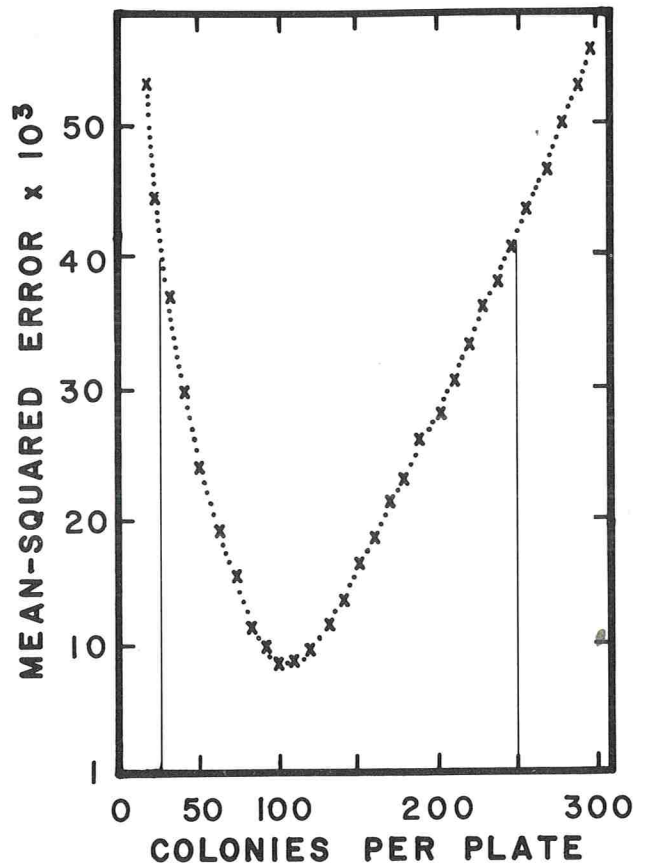


Figure 1. The mean-squared error curve which incorporates the bias and the variability of the colony counts and is used to express the optimum limits for counting colonies. The two fine vertical lines define error terms associated with colony counts of 25 and of 250 colonies per plate.

A set of optimum limits can be determined by placing a horizontal line across the mean-squared-error curve of Fig. 1. If a two-fold dilution was desired, the optimum limits were approximately 70-140 colonies/plate. A five-fold dilution series would have optimum limits of from 40-200 colonies per plate. In a ten-fold dilution series, as is currently used by most bacteriologists, the optimum limits were between 25-250 colonies/plate. Thus these data would indicate that 25-250 colonies/

TABLE 2. Six examples, selected at random, of "diluted count" raw data.

| Dilution number | Dilution factor | Expt. 1 Sample 3 | Expt. 2 Sample 2 | Expt. 2 Sample 11 | Expt. 2 Sample 16 | Expt. 3 Sample 17 | Expt. 3 Sample 20 |
|-----------------|-----------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|
| 1               | 1: 1.00         | 860.00           | 718.33           | 776.33            | 346.33            | 447.00            | 460.67            |
| 2               | 1: 1.40         | 806.00           | 537.67           | 601.33            | 261.00            | 332.00            | 368.67            |
| 3               | 1: 1.96         | 668.67           | 366.67           | 437.67            | 197.33            | 260.67            | 258.33            |
| 4               | 1: 2.74         | 566.00           | 311.00           | 323.00            | 131.33            | 184.33            | 166.00            |
| 5               | 1: 3.84         | 390.67           | 208.33           | 257.00            | 98.00             | 131.33            | 138.00            |
| 6               | 1: 5.38         | 362.67           | 147.00           | 196.67            | 86.33             | 84.00             | 96.33             |
| 7               | 1: 7.53         | 245.67           | 110.33           | 143.00            | 50.67             | 71.67             | 72.00             |
| 8               | 1: 10.54        | 231.33           | 83.00            | 96.00             | 41.67             | 45.67             | 62.33             |
| 9               | 1: 14.76        | 206.00           | 57.33            | 65.00             | 28.67             | 39.33             | 38.00             |
| 10              | 1: 20.66        | 188.33           | 46.33            | 48.00             | 23.67             | 24.33             | 23.33             |
| 11              | 1: 28.93        | 38.00            | 32.67            | 38.33             | 15.67             | 19.00             | 19.00             |
| 12              | 1: 40.50        | 22.67            | 24.33            | 33.33             | 11.00             | 12.67             | 8.33              |
| 13              | 1: 56.69        | 16.67            | 16.33            | 18.67             | 7.00              | 10.67             | 7.67              |
| 14              | 1: 79.37        | 13.67            | 11.33            | 12.33             | 3.00              | 4.33              | 6.67              |
| 15              | 1:111.12        | 7.67             | 8.67             | 6.33              | 2.67              | 4.00              | 4.33              |

TABLE 3. *The diluted counts and their corresponding mean-squared errors.*

| Diluted Counts | Mean-squared errors |
|----------------|---------------------|
| 20             | 44,941.64           |
| 30             | 37,051.62           |
| 40             | 30,132.70           |
| 50             | 24,184.88           |
| 60             | 19,208.15           |
| 70             | 15,202.52           |
| 80             | 12,167.99           |
| 90             | 10,104.59           |
| 100            | 9,012.63            |
| 110            | 8,893.04            |
| 120            | 9,747.36            |
| 130            | 11,577.71           |
| 140            | 13,901.29           |
| 150            | 16,235.92           |
| 160            | 18,585.57           |
| 170            | 20,954.82           |
| 180            | 23,348.86           |
| 190            | 25,773.50           |
| 200            | 28,235.14           |
| 210            | 30,740.83           |
| 220            | 33,298.19           |
| 230            | 35,915.46           |
| 240            | 38,601.52           |
| 250            | 41,365.82           |
| 260            | 44,201.97           |
| 270            | 47,096.98           |
| 280            | 50,050.84           |
| 290            | 53,063.49           |
| 300            | 56,135.05           |

plate is more appropriate than the presently accepted range (4) of from 30-300 colonies/plate.

### DISCUSSION

The principal sources of variation in dilution-plate counting methods have been discussed by a number of investigators (9,11,23,24,25); analyst errors can be especially significant (9,11,12,15,21). For nondairy foods, sampling (18) and plating (19) procedures also are critical. Errors caused by factors other than those examined here are inherent in all dilution-plate counting methods, and our study was aimed solely at determining the most suitable number of colonies on plates for counting. We attempted to reduce other errors by using different analysts to obtain three different sets of data, by using standardized dilution, plating and counting procedures (3) and by collecting data on 65 different samples. The greatest errors introduced by our procedure probably were dilution errors (22); these errors might have been cumulative, in which case, the optimum counting range may have been skewed to slightly higher counts than they should have been.

The optimum counting range, for a 10-fold dilution series of raw milk, was 25-250 colonies per plate. By substituting 25-250 for the presently accepted range for countable plates of 30-300 colonies per plate (4), the number of colonies that need counting could be reduced. Fruin et al. (14) recently reported that, "Plates having 20-200 colonies were as suitable for counting as plates having 30-300 colonies and were counted with a time saving of 27%." Thus, our results (Table 3 and Fig. 1) reinforce their conclusions (14) and pinpoint the

optimum range of 25-250 colonies/plate. Adoption of the counting range of 25-250 colonies/plate should result in time saving, less analyst fatigue and better analyst performance.

The data presented herein are not necessarily applicable to other systems. For automated equipment (10), the optimum counting range may well vary with the instrument, particle (colony) size limits, range of colony sizes, etc. Furthermore, even if automation is not used, appropriate numbers of colonies that should be on a countable plate can vary widely, depending on many other variables. With soil fungi, for example, maxima of from 25-100 colonies per plate have been suggested (17). Coliform analyses demand another range (24). Many of these and other recommended ranges should be examined by using comprehensive sets of data such as those used in our study. The statistical methods developed to determine optimum counting ranges (27) should be applicable to any set of data, whether obtained by the aided eye or an automated piece of equipment.

The recommendations presented herein would not alter, in any respect, plate count limits for standards. Whether or not the number of countable colonies on a plate is representative of the number originally in the sample or their ability to form colonies is another point. There are other means of increasing observed colony counts (28). The relationships of colony counting methods to other methods, such as MPN techniques (24), also remains unchanged.

Results of our studies should encourage cautious use of purely theoretical data based on the assumption that "an important characteristic of a Poisson series is that the standard deviation is equal to the square root of the mean of the distribution, and therefore the count is, in itself, a measure of the precision of the test" (13). Theoretical 95% confidence intervals (13) assume a configuration quite different from the curve that we obtained under practical conditions of using actual colony counts (see also 30). Because of the many errors inherent in dilution-plate counts, actual sets of data must be analyzed to determine the most suitable number of colonies on plates for counting.

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## Collaborative Evaluation of the Plate Loop Technique for Determining Viable Bacterial Counts in Raw Milk

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### ABSTRACT

A collaborative evaluation of the plate loop count technique was undertaken involving 13 laboratories with 29 technologists and 27 plate loops. The log variance of the Plate Loop Count (PLC) was 0.007, compared with a log variance of 0.001 for the reference Standard Plate Count (SPC), both of which fell within the tolerance limit of 0.012 considered acceptable for reproducibility by *Standard Methods*. The overall  $\log_{10}$  mean PLC (1.975) compared favourably with the  $\log_{10}$  mean SPC (1.960). Despite this overall image of precision and accuracy of the PLC, comparative data between individual technicians and/or loops was significantly different. Factors which contributed to this inaccuracy and imprecision of the PLC included improper loop calibration and significant variation in technique not only between analysts but also by individual analysts between loops.

The Plate Loop Count was originally described as a smear culture technique in which a wire loop was used to spread a calibrated volume of milk onto an agar plate surface as a means of measuring viable bacterial numbers (3). Rydzewski (6) adopted this method in 1937 for determining bacterial survival after thermal destruction during pasteurization. Myers and Pence (5) used the calibrated loop to inoculate oval test tubes, containing tempered, sterile agar, for calculating bacterial counts of pasteurized milk samples. Some 19 years later, Donnelly et al. (4) confirmed the equivalence of bacterial counts in raw milk determined by either the oval tube or Standard Plate Count (SPC) technique. Thompson et al. (9) further modified the loop technique of Myers and Pence for use in a pour plate technique. These investigators reported that their Plate Loop Count (PLC) gave equivalent results to the SPC.

We are aware of only two other published studies of the PLC since its introduction in 1960. Tatini et al. (8) found the PLC underestimated the bacterial population in raw milk relative to the SPC when the bacterial density of the milk exceeded  $10^5$  per ml. Wright et al. (12) reported similar results and suggested the  $SPC = PLC^{1.04}$ . Olsen and Richardson, in a report describing a flooded plate loop procedure presented at the 72nd annual meeting of the American Dairy Science Association in 1977, indicated that the PLC had never been subjected to a collaborative evaluation and doubted that it could meet the statistical requirements specified by Brazis et al. (1).

As part of a proficiency testing program, a collaborative study of the PLC was conducted involving

13 laboratories within the Ontario Ministry of Health, Laboratory Services Branch. A total of 29 technologists and 27 plate loops were comparatively evaluated.

### MATERIALS AND METHODS

#### *Sample preparation and distribution*

On the day of distribution, 2500 ml of fresh raw milk from 60 producers was pooled, heated to 85 C for 30 min with constant stirring, rapidly cooled, dispensed in 100-ml amounts into sterile, screw-capped, plastic jars and refrigerated. An NSB (2) suspension of *Staphylococcus aureus*, adjusted to a density of  $1 \times 10^7$  per ml in NSB was dispensed (3-5 ml) into sterile bijou bottles. All samples were refrigerated (4 C) until packed for shipping that same afternoon in foamed plastic insulated boxes with freezer packs. A set of instructions and a data sheet were enclosed with each sample. Each package was shipped to the respective participating laboratories by courier service to ensure delivery the following day. Ten packages were set aside at room temperature for preparing the Standard Plate Count controls.

#### *Preparation of simulated raw milk and analytical instructions*

Upon receipt of the packages, the Head Technologist in each laboratory was instructed to prepare the simulated raw milk samples by carefully adding 1.0 ml of the well-mixed NSB culture to the jar of milk. Each technologist who routinely performed the Plate Loop Count was instructed to thoroughly mix the sample and to plate loop 10 replicates from the simulated raw milk sample with each plate loop routinely used (up to a maximum of three loops) in their respective laboratories. All inoculated plates were to be incubated at 32 C for  $48 \pm 4$  h. The bacterial counts were recorded on the data sheets and returned to the authors for analysis.

#### *Standard Plate Count control*

The day after the proficiency samples were shipped, 10 simulated raw milk samples were prepared as described and analysed by the authors using the Standard Plate Count technique with five replicates per sample. All dilutions were made in phosphate buffered saline solution, pH 7.0. Standard Methods agar (BBL) was prepared in the Agarmatic Bench-Top Agar Sterilizer (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey) and dispensed in 12-15 ml volumes per pour plate. The plates were incubated for  $48 \pm 4$  h at 32 C before counting. The  $\log_{10}$  mean of the SPC results of these 10 reference samples was regarded as the true bacterial density of the simulated raw milk. A sterility check of the pooled, uninoculated raw milk was made using duplicate pour plates with one ml inocula.

#### *Statistical analysis*

The PLC data from each laboratory, and the reference SPC results were converted to  $\log_{10}$  values and examined as recommended by the United States Public Health Service (10) and the American Public Health Association (1). Each technician's results were evaluated comparatively against loops and against results of other technicians within their respective laboratories. Homogeneity of variance (Bartlett's test) was considered a prerequisite to further statistical evaluation by Analysis of Variance (ANOVA) or Student's t-test (11).

## RESULTS

All laboratories received their samples within 24 h of shipment. The variance of the SPC results for the 10 reference samples was found to be homogeneous and ANOVA was not significant ( $\alpha = 0.05$ ). The  $\log_{10}$  mean ( $\bar{X}$ ) of the SPC was 1.960 per ml with a standard deviation (SD) of 0.034 and was regarded as the true bacterial density of the sample.

The overall  $\log_{10} \bar{X}$  of the raw PLC data was 2.020 with a SD of 0.140. Although there were no outlying data, the frequency distribution of the individual observations revealed a bimodal curve (Fig. 1), suggesting measurements from two separate populations. The extraneous observations which formed the much smaller second population were exclusively attributed to technician 1 in laboratory B, technicians 1, 2, 3 and 4 in laboratory K and technician 3, loop 1 in laboratory L and were rejected. Single spurious observations by technician 3 with loop 3 in laboratory E and technician 5 with loop 2 in laboratory K were Winsorized (11).

Table 1 illustrates the comparative evaluation of the technicians using the edited PLC data. Overall, the  $\log_{10} \bar{X}$  PLC was 1.975 with a SD of 0.082. Multi-mean analyses of the PLC data, where applicable, showed significant differences between technicians using certain loops within each of the following laboratories: B (loop 1); C (loops 1 and 2); D (loop 1); E (loop 3); F (loop 2); G (loops 1 and 2); J (loop 2); and M (loops 1, 2 and 3).

Table 2 shows the statistical summary of the comparative evaluation of the PLC data by each loop within the individual laboratories. No multi-mean

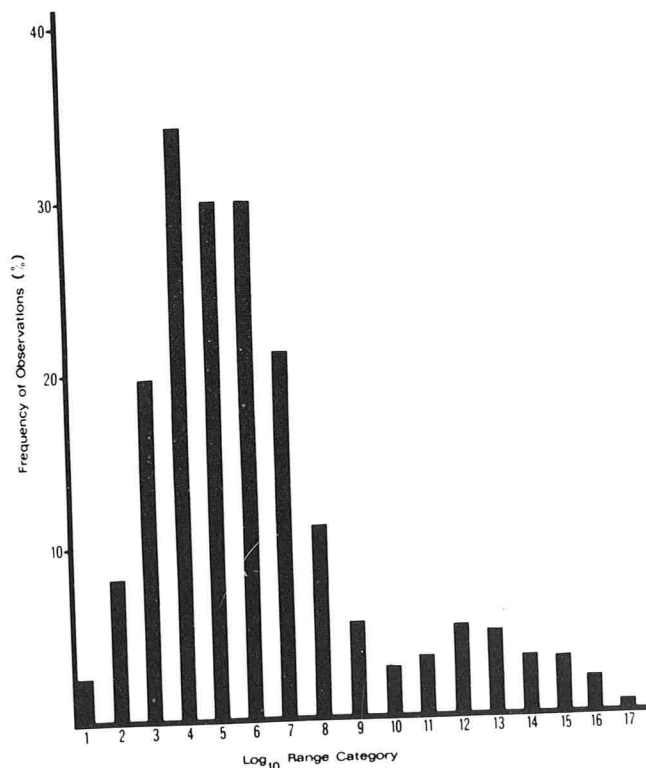


Figure 1. Frequency distribution of Plate Loop Counts.

comparison tests of loop performance were made for the PLC results reported by the analysts in laboratories A and B as only one loop was used in each laboratory. Also, multi-mean comparison tests of loop performance were not applied to the data reported by technician 1, laboratory D as there were significant differences in variance by this technician between loops. Multi-mean analysis of the remaining data revealed some significant differences in loop accuracy. Relative to the true mean, loops 1 and 3 in laboratory E and loop 1 in laboratory F were significantly higher; loop 2 in laboratory H and loop 1 in laboratory I were significantly lower.

Table 3 illustrates a statistical comparison of the edited PLC data with the reference SPC as recommended by Brazis et al. (1). The overall difference between the  $\log_{10} \bar{X}$  PLC (1.975) and  $\log_{10} \bar{X}$  mean SPC (1.960) was 0.015, meeting the acceptability requirement of being less than 0.036; however, certain individual laboratories, technicians and loops did not meet this acceptability criterion: Laboratory A, all technicians; laboratory B, technicians 1 and 3; laboratory C, technician 1 both loops; laboratory E, all technicians, loops 1 and 3 and technician 3, loop 2; laboratory F, both technicians, both loops; laboratory G, technician 2, both loops; laboratory H, both loops; laboratory I, loop 1; laboratory J, technician 3 both loops; laboratory K, loop 2; laboratory L, technician 1, loop 2 and technician 2, loops 1 and 2; laboratory M, technician 1, all loops, technician 2, loop 1 and technician 3, loop 1.

## DISCUSSION

Results of this collaborative study suggest that the PLC technique has the potential of providing measurements of bacterial density in raw milk as reasonably accurate as by the SPC. The overall log variance of 0.007 for the PLC was within the tolerance limit of 0.012 considered acceptable for reproducibility for the SPC. Furthermore, the overall difference between the  $\log_{10} \bar{X}$  PLC and the  $\log_{10} \bar{X}$  SPC was 0.015, within the acceptable limit of 0.036; however, this collaborative study revealed inconsistent PLC techniques which were not acceptable, not only by different technicians using the same loop but also by the same technicians using different loops within the same laboratory. These technologists need to review the described PLC procedure to re-establish uniform timing of loop sampling such that each downward and upward cycle is at a rate of 55-60 beats per minute (1). Removing the loop too slowly results in a lower volume retention, while too quick or jerky removal of the loop causes more than 0.001 ml of milk to adhere.

Some variation in the calibration of the loops was also observed. All loops used in this study were originally obtained from the same manufacturer (Johnson, Matthey and Mallory Ltd., Toronto, SL-503); however, many of the loops had been in routine use for some months. Although the loops had been manufactured to specification, there was no positive assurance of

TABLE 1. Comparative evaluation of technicians in Plate Loop Count performance.

| Laboratory       | Loop | n <sup>a</sup> | Technician   |                 |           |       |                 |       | Analysis of variance |
|------------------|------|----------------|--------------|-----------------|-----------|-------|-----------------|-------|----------------------|
|                  |      |                | 1            |                 | 2         |       | 3               |       |                      |
|                  |      |                | $\bar{X}^b$  | SD <sup>c</sup> | $\bar{X}$ | SD    | $\bar{X}$       | SD    |                      |
| A                | 1    | 30             | 1.897        | 0.054           | 1.868     | 0.069 | 1.918           | 0.047 | NS <sup>d</sup>      |
| B                | 1    | 30             | 2.017        | 0.051           | 1.960     | 0.042 | 2.006           | 0.032 | Se                   |
| C                | 1    | 20             | 2.030        | 0.047           | 1.947     | 0.028 | ND <sup>f</sup> |       | S                    |
|                  | 2    | 20             | 2.058        | 0.040           | 1.988     | 0.055 | ND              |       | S                    |
| D                | 1    | 20             | 1.931        | 0.026           | 1.976     | 0.036 | ND              |       | S                    |
|                  | 2    | 20             | 1.949        | 0.053           | 1.970     | 0.046 | ND              |       | NS                   |
| E                | 1    | 30             | 2.063        | 0.058           | 2.076     | 0.055 | 2.019           | 0.057 | NS                   |
|                  | 2    | 30             | 1.980        | 0.062           | 1.948     | 0.046 | 1.998           | 0.060 | NS                   |
|                  | 3    | 30             | 2.032        | 0.053           | 2.070     | 0.034 | 2.095           | 0.034 | S                    |
| F                | 1    | 20             | 2.055        | 0.038           | 2.075     | 0.045 | ND              |       | NS                   |
|                  | 2    | 20             | 1.919        | 0.062           | 2.005     | 0.050 | ND              |       | S                    |
| G                | 1    | 20             | 1.939        | 0.066           | 1.813     | 0.056 | ND              |       | S                    |
|                  | 2    | 20             | 1.992        | 0.051           | 1.918     | 0.074 | ND              |       | S                    |
| H                | 1    | 10             | 1.910        | 0.035           | ND        |       | ND              |       | ND                   |
|                  | 2    | 10             | 1.828        | 0.053           | ND        |       | ND              |       | ND                   |
| I                | 1    | 10             | 1.839        | 0.050           | ND        |       | ND              |       | ND                   |
|                  | 2    | 10             | 1.927        | 0.054           | ND        |       | ND              |       | ND                   |
| J                | 1    | 30             | 1.992        | 0.047           | 1.932     | 0.047 | 2.013           | 0.105 | NA <sup>g</sup>      |
|                  | 2    | 30             | 1.973        | 0.052           | 1.924     | 0.044 | 1.889           | 0.093 | S                    |
| K                | 1    | 10             | 1.965        | 0.057           | ND        |       | ND              |       | ND                   |
|                  | 2    | 10             | 2.008        | 0.060           | ND        |       | ND              |       | ND                   |
| L                | 1    | 30             | 1.997        | 0.066           | 2.017     | 0.072 | 1.975           | 0.053 | NS                   |
|                  | 2    | 30             | 2.097        | 0.052           | 2.003     | 0.043 | 1.965           | 0.075 | S                    |
|                  | 3    | 20             | Not Included |                 | 1.979     | 0.044 | 1.983           | 0.056 | NS                   |
| M                | 1    | 30             | 2.017        | 0.065           | 1.911     | 0.069 | 1.912           | 0.044 | S                    |
|                  | 2    | 30             | 2.064        | 0.038           | 1.990     | 0.053 | 1.959           | 0.053 | S                    |
|                  | 3    | 30             | 2.048        | 0.049           | 1.952     | 0.057 | 1.937           | 0.042 | S                    |
| A-M <sup>e</sup> | 27   | 600            | 1.975        | 0.082           |           |       |                 |       |                      |

<sup>a</sup>Total No. of observations.<sup>b</sup>Log<sub>10</sub> mean of 10 replicates.<sup>c</sup>Log<sub>10</sub> standard deviation.<sup>d</sup>Not significant ( $\alpha = 0.05$ ).<sup>e</sup>Significant ( $\alpha = 0.05$ ).<sup>f</sup>Not done.<sup>g</sup>Not applicable as variances were not homogeneous.

TABLE 2. Comparative evaluation of loops for Plate Loop Count performance.

| Laboratory | Technician | n <sup>a</sup> | Loop        |                 |           |       |           |       | Analysis of variance |
|------------|------------|----------------|-------------|-----------------|-----------|-------|-----------|-------|----------------------|
|            |            |                | 1           |                 | 2         |       | 3         |       |                      |
|            |            |                | $\bar{X}^b$ | SD <sup>c</sup> | $\bar{X}$ | SD    | $\bar{X}$ | SD    |                      |
| A          | 1          | 10             | 1.897       | 0.054           |           |       |           |       |                      |
|            | 2          | 10             | 1.868       | 0.069           |           |       | ND        |       | ND                   |
|            | 3          | 10             | 1.918       | 0.047           |           |       |           |       |                      |
| B          | 1          | 10             | 2.017       | 0.051           |           |       |           |       |                      |
|            | 2          | 10             | 1.960       | 0.042           |           |       | ND        |       | ND                   |
|            | 3          | 10             | 2.006       | 0.032           |           |       |           |       |                      |
| C          | 1          | 20             | 2.030       | 0.047           | 2.058     | 0.040 |           |       | NS <sup>d</sup>      |
|            | 2          | 20             | 1.947       | 0.028           | 1.988     | 0.055 |           |       | NS                   |
|            | 1,2        | 40             | 1.989       | 0.057           | 2.023     | 0.059 |           |       | NS                   |
| D          | 1          | 20             | 1.931       | 0.026           | 1.949     | 0.053 |           |       | NA <sup>g</sup>      |
|            | 2          | 20             | 1.976       | 0.036           | 1.970     | 0.046 |           |       | NS                   |
|            | 1,2        | 40             | 1.953       | 0.038           | 1.960     | 0.049 |           |       | NS                   |
| E          | 1          | 30             | 2.063       | 0.058           | 1.980     | 0.062 | 2.032     | 0.053 | Se                   |
|            | 2          | 30             | 2.076       | 0.056           | 1.948     | 0.046 | 2.070     | 0.034 | S                    |
|            | 3          | 30             | 2.019       | 0.057           | 1.998     | 0.060 | 2.095     | 0.034 | S                    |
|            | 1,2,3      | 90             | 2.052       | 0.060           | 1.976     | 0.058 | 2.065     | 0.047 | S                    |
| F          | 1          | 20             | 2.055       | 0.038           | 1.919     | 0.062 |           |       | S                    |
|            | 2          | 20             | 2.075       | 0.045           | 2.005     | 0.050 |           |       | S                    |
|            | 1,2        | 40             | 2.065       | 0.041           | 1.962     | 0.070 |           |       | S                    |
| G          | 1          | 20             | 1.939       | 0.066           | 1.992     | 0.051 |           |       | NS                   |
|            | 2          | 20             | 1.813       | 0.056           | 1.918     | 0.074 |           |       | S                    |
|            | 1,2        | 40             | 1.876       | 0.088           | 1.955     | 0.073 |           |       | S                    |
| H          | 1          | 20             | 1.910       | 0.035           | 1.828     | 0.053 |           |       | S                    |
| I          | 1          | 20             | 1.839       | 0.050           | 1.927     | 0.054 |           |       | S                    |
| J          | 1          | 20             | 1.992       | 0.047           | 1.972     | 0.052 |           |       | NS                   |

|   |       |    |       |       |       |       |              |       |    |
|---|-------|----|-------|-------|-------|-------|--------------|-------|----|
|   | 2     | 20 | 1.932 | 0.047 | 1.924 | 0.044 | ND           | NS    |    |
|   | 3     | 20 | 2.013 | 0.105 | 1.889 | 0.092 | ND           | S     |    |
|   | 1,2,3 | 60 | 1.979 | 0.077 | 1.928 | 0.073 | ND           | S     |    |
| K | 1     | 20 | 1.965 | 0.057 | 2.008 | 0.059 | ND           | NS    |    |
| L | 1     | 20 | 1.997 | 0.066 | 2.097 | 0.052 | Not included | S     |    |
|   | 2     | 30 | 2.017 | 0.072 | 2.003 | 0.043 | 1.979        | 0.044 | NS |
|   | 3     | 30 | 1.975 | 0.053 | 1.965 | 0.075 | 1.983        | 0.056 | NS |
|   | 1,2,3 | 80 | 1.996 | 0.065 | 1.984 | 0.062 | 1.981        | 0.049 | NS |
| M | 1     | 30 | 2.017 | 0.065 | 2.064 | 0.038 | 2.048        | 0.049 | NS |
|   | 2     | 30 | 1.911 | 0.069 | 1.990 | 0.053 | 1.952        | 0.057 | S  |
|   | 3     | 30 | 1.912 | 0.044 | 1.959 | 0.053 | 1.937        | 0.042 | NS |
|   | 1,2,3 | 90 | 1.947 | 0.077 | 2.004 | 0.065 | 1.979        | 0.069 | S  |

a,b,c,d,e,f and g Refer to Table 1.

TABLE 3. Statistical comparison of accuracy of Plate Loop Count with Standard Plate Count.

| Laboratory | Technician | Log <sub>10</sub> $\bar{X}$ SPC — Log <sub>10</sub> $\bar{X}$ PLC <sup>a</sup> |                 |       |       |
|------------|------------|--------------------------------------------------------------------------------|-----------------|-------|-------|
|            |            | Loop                                                                           |                 |       |       |
|            |            | 1                                                                              | 2               | 3     | All   |
| A          | 1          | .063                                                                           | ND <sup>b</sup> | ND    | ND    |
|            | 2          | .092                                                                           | ND              | ND    | ND    |
|            | 3          | .042                                                                           | ND              | ND    | ND    |
|            | 1,2,3      | .065                                                                           | ND              | ND    | .065  |
| B          | 1          | .057                                                                           | ND              | ND    | ND    |
|            | 2          | .002                                                                           | ND              | ND    | ND    |
|            | 3          | .045                                                                           | ND              | ND    | ND    |
|            | 1,2,3      | .034                                                                           | ND              | ND    | .034  |
| C          | 1          | .070                                                                           | .098            | ND    | .030  |
|            | 2          | .013                                                                           | .028            | ND    | .064  |
|            | 1,2        | .030                                                                           | .064            | ND    | .047  |
| D          | 1          | .029                                                                           | .011            | ND    | .006  |
|            | 2          | .016                                                                           | .001            | ND    | .001  |
|            | 1,2        | .006                                                                           | .005            | ND    | .003  |
| E          | 1          | .103                                                                           | .020            | .072  | .067  |
|            | 2          | .115                                                                           | .012            | .110  | .074  |
|            | 3          | .059                                                                           | .038            | .129  | .075  |
|            | 1,2,3      | .093                                                                           | .016            | .103  | .072  |
| F          | 1          | .115                                                                           | .045            | ND    | .081  |
|            | 2          | .095                                                                           | .041            | ND    | .033  |
|            | 1,2        | .106                                                                           | .004            | ND    | .058  |
| G          | 1          | 0.021                                                                          | 0.032           | ND    | 0.006 |
|            | 2          | 0.147                                                                          | 0.042           | ND    | 0.091 |
|            | 1,2        | 0.079                                                                          | 0.003           | ND    | 0.040 |
| H          | 1          | 0.050                                                                          | 0.134           | ND    | 0.089 |
| I          | 1          | 0.121                                                                          | 0.033           | ND    | 0.075 |
| J          | 1          | 0.032                                                                          | 0.013           | ND    | 0.023 |
|            | 2          | 0.028                                                                          | 0.036           | ND    | 0.032 |
|            | 3          | 0.053                                                                          | 0.071           | ND    | 0.005 |
|            | 1,2,3      | 0.020                                                                          | 0.030           | ND    | 0.004 |
| K          | 1          | 0.005                                                                          | 0.053           | ND    | 0.030 |
| L          | 1          | 0.029                                                                          | 0.139           | .024  | 0.053 |
|            | 2          | 0.056                                                                          | 0.043           | .019  | 0.040 |
|            | 3          | 0.015                                                                          | 0.005           | .023  | 0.015 |
|            | 1,2,3      | 0.034                                                                          | 0.067           | .006  | 0.037 |
| M          | 1          | 0.057                                                                          | 0.104           | 0.088 | 0.084 |
|            | 2          | 0.049                                                                          | 0.030           | 0.008 | 0.008 |
|            | 3          | 0.048                                                                          | 0.005           | 0.023 | 0.024 |
|            | 1,2,3      | 0.011                                                                          | 0.047           | 0.022 | 0.020 |
| A-M        | 29         |                                                                                |                 |       | 0.015 |

<sup>a</sup>Acceptable difference is  $\leq 0.036$ .

<sup>b</sup>Not done.

maintaining the same loop dimensions after repeated use (6). The original calibration of the loops described by Burri (3) was gravimetrically determined, using loops which held 0.5 to 1 mg of water. Burri suggested, however, that the loops could be calibrated by comparing loop counts of bacteria with standard plate counts. While this approach for checking loop calibration is simple in concept, our collaborative study has shown how difficult this is to achieve in practice. Loops which pick up such small volumes of liquid as 0.001 ml are difficult to calibrate because of operational volume transfer errors (6). Furthermore, the volume of sample picked up by the loop is influenced by the surface tension of the liquid (3) and this must be carefully controlled in any comparative study between the PLC and the SPC. A simple method of checking the loop calibration using No. 53 and No. 54 twist drills has been suggested (1). The loops should fit over the shank of a No. 54 but not over the shank of a No. 53 twist drill. In addition to this method of routinely checking the calibration of the loops, we would suggest that the loops be examined microscopically for pitting, roughness of the wire and build-up of milk deposits, all of which may contribute to volume transfer errors.

This collaborative evaluation of the Plate Loop Count demonstrated importance of ensuring proper loop calibration, and the significance of maintaining and reviewing technical proficiency with this procedure.

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## Md. Food Processing Technique Conserves Energy and Money

University of Maryland researchers have developed an energy and money-saving method of preserving the "fresh" quality of raw or partially processed foods.

The process, known as "Gaspak," treats foods with gases to retard bacterial growth and food aging. It results in energy savings of up to 80 percent of the total now used to process foods from the field to the table.

"Gaspak" involves placing a food substance, sliced apples for example, in a chamber and removing all air before treating the food with gases, such as carbon monoxide and sulfur dioxide. The treated food is then packaged in a sterile container filled with gas which will continue to

inhibit germ growth and structural deterioration for long periods of time.

"Gaspak" was conceived by Dr. Amihud Kramer, a professor of food science and a scientist with the University of Maryland's Agricultural Experiment Station. He directs the station's preservation of fresh solid foods by gas exchange project, which is funded by the U.S. Department of Energy and the station.

Dr. Kramer says a technical review panel reacted very favorably to 30 to 250-day-old "Gaspak" food samples it inspected, smelled, and tasted earlier this fall.

Although most research in the two-year project has focused on potatoes and apples, studies have been made with beef patties, mushrooms, strawberries, salad mix, green beans, grapes, chopped lettuce, and sliced peaches.

The U.S. Department of Defense has indicated an interest in the project not only in as it relates to such foods as potato strips for french fries, but also for the storage of whole potatoes for several months at room temperatures. The military also has expressed an interest in the use of some type of GASPAK pretreatment for such highly perishable products as lettuce which need to be shipped in large quantities for long distances.

Research estimates - show that "Gaspak" energy use is almost half of that of canning and freezing. The total energy consumed in processing, marketing, and home preparation of a "Gaspak" produce was estimated by University of Maryland researchers to be 7,962 BTUs per pound, compared with over 14,000 BTUs per

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## Bacteriological and Temperature Survey of Ginger Beef Pot Roast Production at a Central Food Preparation Facility

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### ABSTRACT

A time-temperature and bacteriological survey of roast beef production at a Central Food Processing Facility was undertaken to identify and eliminate food preparation practices which had previously occasionally led to excessive numbers of *Clostridium perfringens* in roast beef at that facility. *C. perfringens* was not detected in the meat or in additives at any stage of the process, but potentially hazardous conditions which would allow its growth, if present, were identified.

During fiscal year 1978, the United States Department of Defense purchased 9,350,200 lb. of boneless beef rounds for roasting, at a cost of 12,070,061.96 dollars, (personal communication; Defense Personnel Support Center, Philadelphia, PA). The frequency with which roast beef is implicated as a vehicle in foodborne disease outbreaks (1,3,5) warrants close surveillance by the Military Food Service to ensure that it is safely prepared and stored. In the United States, roast beef is probably the most frequently reported vehicle for foodborne disease outbreaks (3), including beef-associated salmonellosis (5) and *Clostridium perfringens* food poisoning (1). From 1974 through 1976, *C. perfringens* was responsible for 37 outbreaks, 1,791 cases of foodborne illness and two deaths in the United States (4). In 1976, *C. perfringens* was responsible for 4.5% of confirmed foodborne disease outbreaks and 14.2% of cases in the United States (4). These figures are probably very conservative, considering the number of outbreaks of unknown etiology (4). Therefore, since *C. perfringens* outbreaks usually involve beef (4), precautions must be taken to prevent its growth during preparation and storage of roast beef.

Microbiological analysis of food samples which were collected daily at an Army Central Food Processing Facility (CFPF) had occasionally resulted in condemnation of roast beef due to the presence of excessive numbers of *C. perfringens* (greater than 1000/g in a single sample and/or greater than 100/g in more than two of each five samples collected). A recent (November, 1978) condemnation of roast beef production at this CFPF due to *C. perfringens* counts greater than 10<sup>5</sup>/g prompted the Surgeon General's Medical Advisory Committee on CFPF's to request an investigation which resulted in the following survey.

The purpose of this study was to identify and eliminate food preparation practices that led to excessive numbers

of *C. perfringens* in roast beef prepared at the Army CFPF in question. This was accomplished by conducting a time-temperature and bacteriological survey of beef, condiments and other additives at each stage during production of ginger beef pot roasts to determine potential opportunities for contamination, survival and multiplication of *C. perfringens*.

### MATERIALS AND METHODS

A time-temperature survey of the thawing, cooking, holding, slicing, panning and freezing processes, and a bacteriological survey of raw and cooked beef roasts, as well as condiments and other additives, during production of ginger beef pot roasts at an Army CFPF were made to determine potential opportunities for contamination, survival and multiplication of *C. perfringens*. Aerobic plate counts (APC) and coliform counts were also made to determine the general microbiological quality of the beef and the effectiveness and sanitation of the process.

#### Central Food Processing Facility (CFPF)

The Army Base CFPF examined was a 20,558-ft<sup>2</sup> facility, which prepared, froze and stored, until needed, most food requirements for dining halls on the base including entrees, vegetables and bakery products. Boneless beef rounds were received frozen in cardboard boxes and immediately refrigerated at 2 C (36 F). The entire production of roasts during this survey was carried out in the usual way, and although workers were aware of the survey they were not given any special instructions.

#### Processing ginger beef pot roast

Approximately 800 lb. of frozen beef rounds, weighing 4 to 6 lb. each, were thawed at 2 C (36 F) for 3 days. Each round was surface-browned in a deep-fat fryer and sprinkled with a mixture of monosodium glutamate, salt and pepper (Fig. 1). Tomato and onion sauce was poured over the surface of each roast just before placing the meat into ovens. Meat was roasted at 176.7 C (350 F) in two convection ovens with rotating shelves. When a roast reached an internal temperature of 62.8 C (145 F), or higher it was removed from the oven and held at ambient temperature (23.9 to 29.4 C) until slicing was completed (up to 190 min). Sliced meat was weighed into foil half steam table trays and covered with hot gravy. Temperature of the gravy ranged from 91.1 C (196 F) initially to 61.7 C (143 F) when added to the last tray of meat. Tray packs were sealed and frozen at -43 C (-45 F) in large walk-in blast freezers.

#### Temperature measurements

Temperature measurements at each process stage (Fig. 1) were made with sanitized Weston dial thermometers (Weston Instrument Inc. Newark, N.J.) calibrated at regular intervals against certified National Bureau of Standards thermometers.

Raw meat during thawing at 2 C (36 F) for 3 days was checked by inserting thermometers into one or two roasts in each of 16 cases of meat. Roasts on the outside edge of each case were checked because they were expected to represent the worst case (highest temperature).

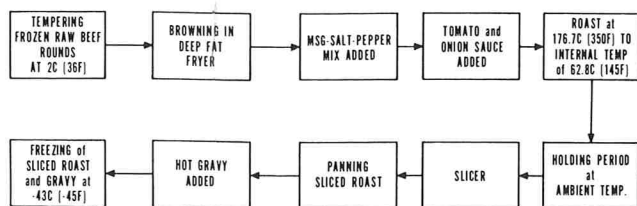


Figure 1. Production sequence of ginger beef pot roast is a central food preparation facility.

Measurements during cooking were made whenever ovens were opened to turn the roasts or to determine doneness. Internal and external temperatures of 5 to 15 roasts selected at random were taken at each time period. During the holding period at ambient temperatures, thermometers were inserted into five roasts selected at random and remained in place until the roasts were sliced. These five roasts were the last to be sliced so that a temperature profile of the entire holding period could be obtained. Temperature readings were made every 15 to 30 min. After slicing and weighing the meat into trays, temperature readings were made at 15- to 20 min intervals. Temperature of the gravy, which was ladled over sliced meat, was measured every 15 to 20 min. Temperature measurements of panned, sliced meat during the freezing stage were made at 30- to 60-min intervals by inserting a thermometer into the corner of five pans selected at random on the freezing racks.

#### Microbiological analyses

Microbiological quality of beef roasts and additives was determined at each process stage (Fig. 1) by analyzing five 50- to 100-g samples for aerobic plate count (APC), total coliforms (TC) and *C. perfringens* (CP) according to standard procedures (7). Samples from five roasts or pans, as appropriate, were collected aseptically, placed in sterile Whirl Pak bags (Scientific Products, Bedford, MA) and refrigerated on ice until tested. Surface samples extending an inch or two into the meat were collected from raw and cooked whole roasts and entire slices were collected from sliced roasts. Swab samples of equipment surfaces were collected by swabbing five, 8-in.<sup>2</sup> areas, as recommended by the U.S. Public Health Service (8). Swabs were analyzed for APC, coliforms, *Staphylococcus aureus* and *C. perfringens*.

#### Media

All media were purchased from Difco Laboratories, Detroit, Michigan. Plate Count Agar was used for APC, Violet Red Bile Agar was used for counting total coliforms and egg yolk-free Tryptose-Sulfate-Cycloserine (TSC) Agar was used for counting *C. perfringens* (7). Swabs were placed directly into cooked meat medium for primary recovery of *C. perfringens* and then subcultured in TSC agar. Baird-Parker agar was used for recovery of *S. aureus* from equipment.

## RESULTS

Figure 1 (flow diagram) shows each stage of the beef pot roast production in the CFPF. Figure 2 is a composite curve of the average internal temperatures of the beef roasts during the entire process. Elapsed time and major stages in the process are denoted by different symbols in the curve. The danger zone (temperature range 7.2 to 60 C, in which the hazard of bacterial growth is greatest) is indicated by the lined area. The shaded area within this zone indicates the temperatures which could support growth of *C. perfringens*. During the thawing stage at 2 C (36 F), for 3 days, the internal temperature of the roasts never exceeded 0 C (32 F). Surface temperature ranged from 0 to 4 C (32-40 F). Browning the surface of roasts by frying each roast for a few minutes was completed in 1 h and did not raise the

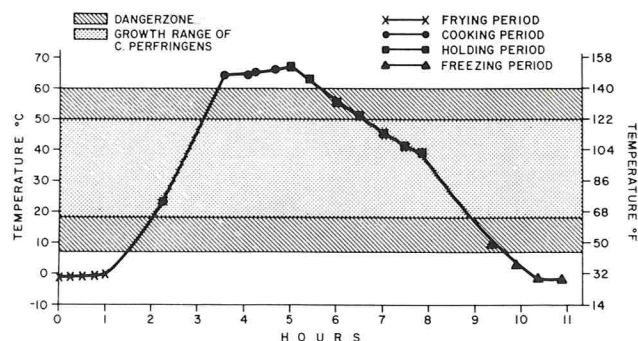


Figure 2. Elapsed time and average internal temperature of ginger beef pot roasts during cooking, holding and freezing periods.

internal temperature. During the cooking stage in dry rotary ovens set at 176.7 C (350 F), the average internal temperatures increased gradually to 62.8 C (145 F), at which time roasts were removed from the oven. All roasts were cooked and removed from ovens within 220 min. After cooking and during the holding and slicing period at ambient temperatures (75-85 F), average internal temperatures of the roasts were within the danger zone, 7.2-60 C (45-140 F), for approximately 4 h which is the maximum time period considered to be safe for cooling foods (6). However, the roasts were in the temperature range for most rapid bacterial growth, 15.6 C (60 F) to 48.9 C (120 F), for 2.5 h, which is not recommended (6). Average internal temperatures of the roasts during holding and slicing were also in the temperature range which supports growth of *C. perfringens*, 18 C (65 F) to 50 C (122 F), for 140 min. The optimum growth temperature for *C. perfringens* is 46 C (114.8 F). Average surface temperatures (not shown) of roasts were within the bacterial growth range in less than 20 min after removal from ovens, and for more than 5.5 h. Sliced meat and gravy, in foil half steam table trays, reached freezing temperatures within 2.5 h after entering the blast freezer (Fig. 2).

Table 1 shows bacterial counts of raw and cooked meats at different stages in the process. Cooking reduced the APC by more than 99.9% from an average of 22,000/g on the raw meat to only 160/g before slicing. Coliforms on raw meat were destroyed by cooking, but were detected in panned meat after slicing, indicating post-cooking contamination. However, only two of the five samples of panned meat tested contained coliforms; one had a coliform count of 500/g and one had a count of 100/g. The remaining three samples had fewer than 100 coliforms per gram (no count at the 1:100 dilution). Freezing reduced the APC to only 40/g, and the average coliform count to only 2/g. Only one out of five frozen samples tested had coliforms and at a count of only 10/g. *C. perfringens* was not detected in the meat at any stage of the process at the lowest dilution counted, and was thus < 100/g in raw and cooked panned meat and < 10/g in cooked frozen meat.

Table 2 shows counts obtained in additives to the ginger beef pot roast and on the slicer. The tomato and onion sauce and the salt-pepper-mono-sodium-

TABLE 1. Microbiology of ginger beef pot roast during processing.

| Bacteria              | Microorganisms per gram          |                      |                  |                      |                      |                      |                 |                      |
|-----------------------|----------------------------------|----------------------|------------------|----------------------|----------------------|----------------------|-----------------|----------------------|
|                       | Raw pot roast<br>(Thawed 3 days) |                      | Cooked pot roast |                      |                      |                      |                 |                      |
|                       | Range                            | Average <sup>a</sup> | Before slicing   |                      | Panned after slicing |                      | Frozen          |                      |
|                       |                                  |                      | Range            | Average <sup>a</sup> | Range                | Average <sup>a</sup> | Range           | Average <sup>a</sup> |
| Aerobic plate count   | 2200 to<br>41000                 | 22000                | < 100 to<br>400  | 160                  | 200 to<br>800        | 460                  | < 100 to<br>100 | 40                   |
| Coliforms             | < 100 to<br>1200                 | 240                  | < 100            | < 100                | < 100 to<br>500      | 120                  | < 10 to<br>10   | 2                    |
| <i>C. perfringens</i> | < 100                            | < 100                | < 100            | < 100                | < 100                | < 100                | < 10            | < 10                 |

<sup>a</sup>Average of 5 samples.

TABLE 2. Microbiology of additives to ginger beef pot roast and of slicer surfaces.

| Sample                               | Average count <sup>a</sup> |                         |                                 |                         |
|--------------------------------------|----------------------------|-------------------------|---------------------------------|-------------------------|
|                                      | Aerobic<br>plate<br>count  | Coliforms               | <i>C.</i><br><i>perfringens</i> | <i>S. aureus</i>        |
| Tomato and onion sauce               | 18000/g                    | < 100/g <sup>c</sup>    | < 100/g                         | — <sup>e</sup>          |
| Salt-pepper-MSG <sup>b</sup> mixture | 75000/g                    | < 100/g                 | < 100/g                         | — <sup>e</sup>          |
| Gravy                                | < 100/g                    | < 100/g                 | < 100/g                         | — <sup>e</sup>          |
| Slicer blade                         | 0.75/in.                   | < 0.25/in. <sup>2</sup> | 0/40 in. <sup>2d</sup>          | < 0.25/in. <sup>2</sup> |
| Slicer platform<br>(cutting surface) | 1.0/in. <sup>2</sup>       | < 0.25/in. <sup>2</sup> | 0/40 in. <sup>2d</sup>          | — <sup>e</sup>          |

<sup>a</sup>Average of 5 samples.

<sup>b</sup>MSG = Monosodium glutamate.

<sup>c</sup>Counts preceded by the symbol "<" were negative at the lowest dilution cultured.

<sup>d</sup>Swabs were cultured directly in cooked meat medium.

<sup>e</sup>Not tested.

glutamate mixture combined, contained nearly 10<sup>5</sup> organisms (APC) per gram, and were added to uncooked roasts just before placing them into the oven. No counts at lowest dilution (1:100) were obtained from gravy because the temperature remained very hot; between 91.1 C (196 F) initially and 62 C (143 F) at the completion of the panning operation. The slicer was very sanitary and had an average of less than 1 organisms per square inch of surface. Surfaces of the slicer were also negative for *C. perfringens* and *S. aureus*.

## DISCUSSION

The bacteriological quality of both raw and cooked beef, and the overall sanitation of the roast beef production in the CFPF was good. Although *C. perfringens* was not detected, the meat was held within its optimum growth range long enough to be potentially hazardous if the organism had been present in large numbers. With a generation time of 8-12 min at its optimal growth temperature, *C. perfringens* could increase 1000 to 30,000-fold in 3 to 4 h (1). Therefore, the major weakness in the operation at the CFPF investigated was the practice of holding cooked meat at ambient temperatures above 21.1 C (70 F). During the last outbreak of *C. perfringens* at this facility, the temperature of the roast beef was reportedly within the

bacterial growth range for 18 h before slicing, at which time the temperature of the meat was 15-16 C (59-60.8 F). This long time at temperatures suitable for bacterial growth was undoubtedly a major factor in finding excessive numbers of *C. perfringens* at that time. Inadequate refrigeration and warm holding for long periods are the two most important factors that contribute to *C. perfringens* foodborne illness outbreaks (2). Recommendations made included quartering of cooked roasts to speed cooling, and refrigeration during the holding period before slicing. Slicing of meat when it is cold will not only inhibit bacterial growth but will also increase yield of the meat.

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# Adhesive Tape Method for Estimating Microbial Load on Meat Surfaces<sup>1</sup>

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## ABSTRACT

Acetate and mylar adhesive tapes were used to estimate microbial loads on surfaces of 60 red meat samples. The conventional method of excision, rinsing and blending of meat was used as a comparison. The mylar tape method was found to provide statistically significant correlation compared with the conventional method. We suggest that when the tape count is  $> \log 2$  CFU/cm<sup>2</sup>, bacterial counts are high on meat surfaces (ca.  $\log 5-7$  CFU/cm<sup>2</sup>); between  $\log 1-2$  CFU/cm<sup>2</sup>, counts are intermediate (ca.  $\log 3-4$  CFU/cm<sup>2</sup>) and  $< \log 1$  CFU/cm<sup>2</sup>, counts are low (ca.  $\log 3$  CFU/cm<sup>2</sup>). The tape method is easy to perform and requires little time and material. With multiple transfers, it may be used to evaluate counts at different incubation temperatures and on different types of agar media.

Workers concerned with food plant sanitation, food spoilage and food preservation need to monitor the microbial load on surfaces of food products and in food processing environments. Baldock (1) reviewed the various methods and procedures for sampling surfaces for microorganisms in relation to food plant sanitation. Vanderzant et al. (13) identified nine ways to evaluate microbial loads on meat and meat products surfaces: skin or meat tissue excision, swabbing, rinsing, direct agar contact, skin-scraping, impression (tape), vacuum, light scattering, radiometric and bioluminescence evaluation and blending with sterile diluent methods. They also listed the following factors that influence selection of test procedures: type of sample, objective of the test, microbial levels expected, presence of bactericidal compounds on surface of sample, resources available for sampling and laboratory analysis, environmental conditions during sampling and precision and accuracy requirements.

Of those procedures and methods, the agar-contact method and impression tape method are said to be the easiest to use, especially in field testing.

Use of adhesive tape for sampling microorganisms from human skin has been reported by Edwards and Hartman (2), Kooyman and Simons (3), Milne and Barnetson (6), Thomas (10), Ulrich (11), Updegraff (12) and Wilson (14). Newfarmer and Robe (7) reported on the effectiveness of a commercial tape-plate system in sampling microorganisms. But adhesive tape for sampling microorganisms from red meat surfaces has not yet been extensively used. This report describes a

simple system for estimating microbial loads of red meat surfaces with an adhesive tape (acetate or mylar) as the sampling device and agar medium as the growth substrate. Results were compared with those from the conventional plate-count method of excised, rinsed and blended and rinsed samples, a method credited with providing accurate microbial counts on meat (4).

## MATERIALS AND METHODS

### *Meat samples*

Sixty meat samples were obtained from the Department of Animal Sciences and Industry, Kansas State University, half of them directly from the wholesale short plate region of the washed carcasses and these are considered fresh samples. Companion wholesale short plate samples were excised, vacuum-packaged and stored for 14 days at 2 C before sampling. These were the stored samples. Samples were excised anterior of the 13th rib and 6.0 to 8.0 cm dorsal to the ventral midline.

### *Acetate adhesive tape procedure*

For the first 40 samples (20 fresh, 20 stored) acetate adhesive tapes (7.9 × 13.1 cm with adhesive area of 7.9 × 12.4 cm; Dynatech Laboratory Inc., Alexandria, VA) were used. After removing the protective paper from the tape, the adhesive side was pressed on the surface of the carcass plate region (Fig. 1) or plate pieces. The adhesive side of the tape has been determined to be free of microorganisms in our laboratory by agar contact method. Contact time on meat was approximately 20 sec. The tape charged with microorganisms was then transferred to sterile plate-count agar (Difco) contained in a rectangular "petri dish" (8.5 × 12.5 cm; Fig. 2). During the experiments we wore disinfected rubber gloves to minimize contamination. After contact time of 5 min, the tape was removed and the plate was then incubated at 32 C for 24 h for mesophilic counts.

For 20 samples, the tape was further transferred to another sterile agar plate. The second agar plate was incubated at 7 C for 10 days for psychrotrophic counts. Preliminary data indicated that the tape could be transferred five times before appreciable reduction of counts on the plate was observed. After incubation, the number of colonies on the plate was counted directly when the number was fewer than 500 per plate and then was reported as log CFU (colony forming units)/cm<sup>2</sup>. When larger numbers were encountered, a template with 160 squares was used to estimate the number of colonies on the plate. At pre-designated intervals the number of organisms in 16 squares was counted. The total number in 16 squares was multiplied by 10 to obtain the total number of CFU/cm<sup>2</sup> on the plate. When the organisms in a square were too numerous to count, an arbitrary "saturation" number of 200 colonies was assigned, so the maximum colonies countable were 32,000. As a comparison, two pieces of meat (32.26 cm<sup>2</sup>) adjacent to the tape sampling area were excised aseptically (19), placed in 100 ml of sterile rinse solution (buffered diluent) (5) for 1 h and then shaken 100 times before the plate count was made (5).

### *Mylar adhesive tape method*

Mylar adhesive tape (4 × 1.31 cm; with adhesive area of 4 × 12.5 cm, Dynatech Laboratory Inc., Alexandria, VA) was tested on 20 meat

<sup>1</sup>Contribution No. 80-13-J, Department of Animal Sciences and Industry, Kansas Agricultural Experiment Station, Manhattan 66506.

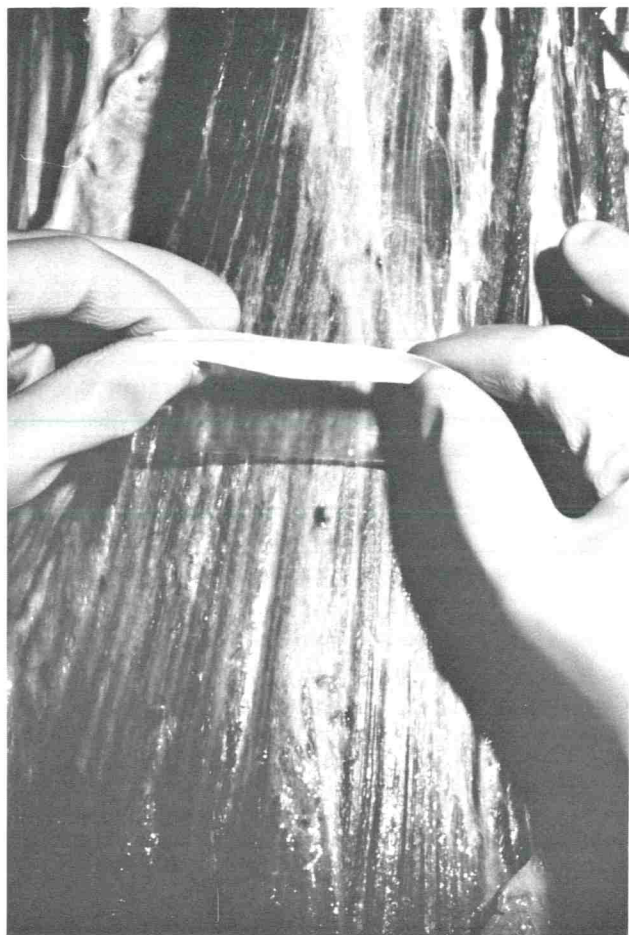


Figure 1. Removing acetate adhesive tape from carcass after sampling of microorganisms.

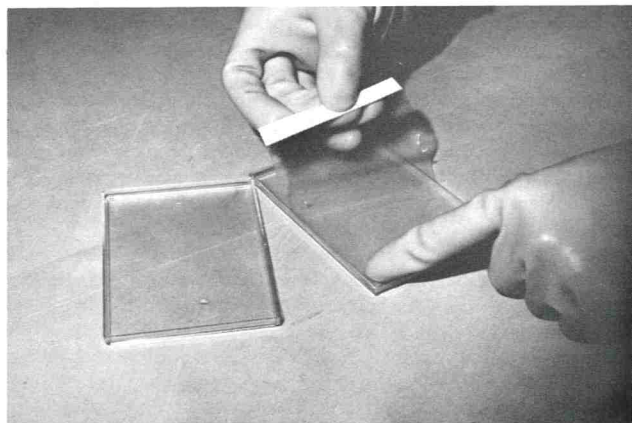


Figure 2. Applying acetate adhesive tape to sterile agar surface. The lid of the rectangular "petri dish" is shown on the left.

samples (10 fresh, 10 stored). The procedure was similar to the acetate tape method except that both the contact time on the meat surface and the agar surface were 1 min each. The counting procedure remained the same. The template used for this study had 75 squares, of which 10 were randomly selected and counted to estimate the number of plates with more than 500 CFU per plate. The multiplication factor was 7.5. For comparison, one piece of meat (32.26 cm<sup>2</sup>) was aseptically excised adjacent to the tape sampling area, placed in 100 ml of rinse solution for 20 min, blended for 5 sec, and shaken 50 times before the plate count was made. In this experiment, both mesophilic and psychrotrophic counts of meat were monitored for the tape method and the conventional method.

## RESULTS AND DISCUSSION

Results of psychrotrophic and mesophilic counts of the 40 (fresh and stored) samples by the acetate adhesive tape method and the conventional method are presented in Fig. 3. Microbial loads were log 0-5 CFU/cm<sup>2</sup> by the conventional rinse method and log 1 to 2 CFU/cm<sup>2</sup> by the tape method. Correlation coefficients between the two methods for 20 paired psychrotrophic counts was 0.64 and for 40 paired mesophilic counts, 0.51 ( $P < 0.01$ ) (8).

In the mylar adhesive tape study (20 samples, fresh and stored), the microbial loads were log 0-7 CFU/cm<sup>2</sup> for the conventional blend rinse method and log 2 to 2 CFU/cm<sup>2</sup> for the tape method. Correlation coefficient between 20 paired psychrotrophic counts was 0.95 and for 20 paired mesophilic counts, 0.90 ( $P < 0.01$ , Fig. 4).

These data indicate that the tape method, especially with mylar adhesive tape, provides a reliable estimate of the microbial load on surfaces of red meat. The data suggest that when the tape count is  $> \log 2$  CFU/cm<sup>2</sup>, bacterial counts are high on meat surfaces (ca. log 5-7 CFU/cm<sup>2</sup>); between log 1-2, counts are intermediate (ca. log 3-4 CFU/cm<sup>2</sup>) and  $< \log 1$  CFU/cm<sup>2</sup>, counts are low

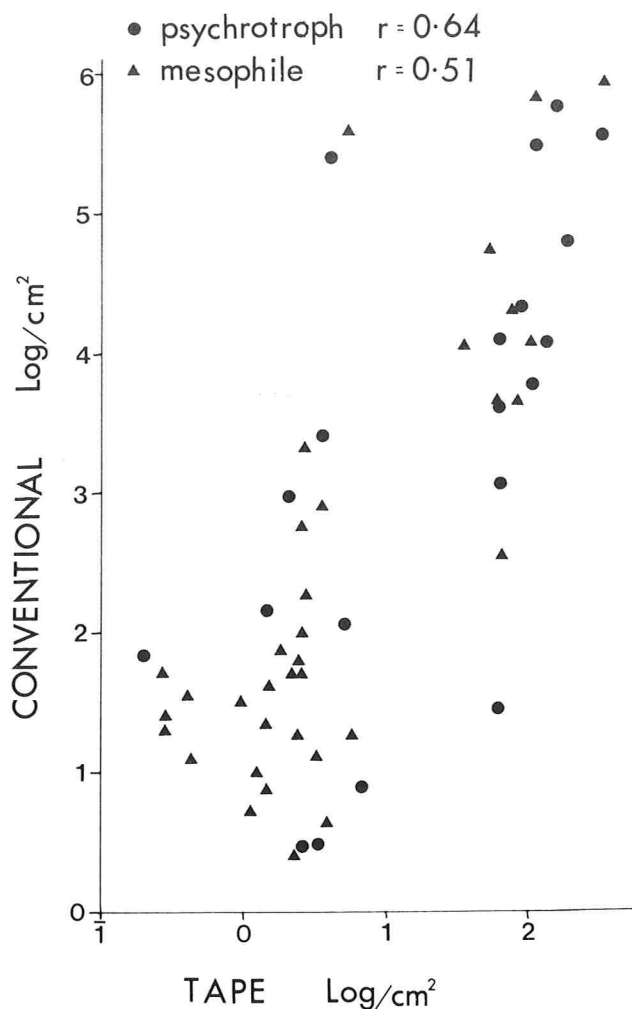


Figure 3. Correlation of the conventional plate-count method and the acetate-adhesive-tape method for psychrotrophic and mesophilic microbial loads on meat surfaces.

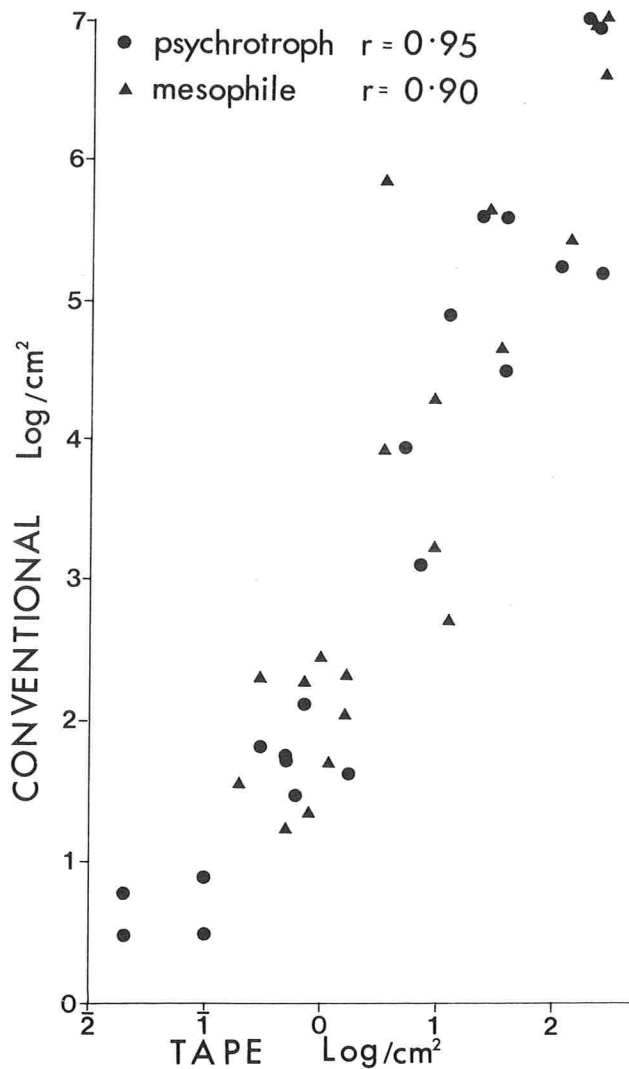


Figure 4. Correlation of the conventional plate count method and the mylar-adhesive-tape method for psychrotrophic and mesophilic microbial loads on meat surfaces.

(ca.  $< \log 3$  CFU/cm<sup>2</sup>).

The difference in correlation coefficients between the two paired studies probably stemmed from the 5-sec blending step used in the conventional method in the mylar tape study and also from increasing mylar-tape contact time on meat surfaces from 20 sec to 1 min which may have increased the number of bacteria adhering to the tape.

The tape method is easy to use and requires less time

and material than the conventional method. With multiple transfers, it may be used to evaluate counts at different incubation temperatures and on different types of agar media. Its flexibility lets it follow the contours of meat surfaces during sampling. Further investigation should show that the tape method is applicable to other surfaces. Variables related to adhesive tape techniques include the nature of the surfaces studied, the effect of temperature, contact time, humidity, pH, electrostatic forces, agar media and perhaps other undetermined factors. As the tape method is easy to use, especially for on-site sampling, further investigation on the usefulness of the method is warranted.

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## A Research Note

# Enterotoxin Production in Milk by Enterotoxigenic *Escherichia coli*<sup>1</sup>

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### ABSTRACT

Three enterotoxigenic strains and one nonenterotoxigenic strain of *Escherichia coli* were grown in milk at an initial pH of 6.5 to 8.5. One strain produced detectable levels of heat-labile enterotoxin in all cultures with an initial pH above 6.5.

Enterotoxigenic *Escherichia coli* are known to produce diarrheal disease in humans by colonizing the anterior region of the small intestine and there producing a heat-labile (LT) and/or a heat-stable (ST) enterotoxin that induces fluid secretion into the intestine (12). It is estimated that an "infectious dose" of  $10^8$  to  $10^9$  viable cells must be consumed in a contaminated food product to cause illness (10). Enterotoxigenic strains have been found in a number of foods, including dairy products (10,13), and at least two outbreaks of gastroenteritis have been traced to dairy products contaminated with enterotoxigenic *E. coli* (8,9). The possibility of enterotoxigenic strains producing enterotoxin during their growth in a food product has been suggested (4) but has not been investigated. We report here that enterotoxin production by *E. coli* growing in milk can occur.

### MATERIALS AND METHODS

#### Bacterial strains

Four strains of *E. coli* used in this study are listed in Table 1. Strains 263 and 1261 were obtained from Dr. Harley Moon, National Animal Disease Center, Ames, IA. Strain 711 was obtained from Dr. Stanley Falkow, University of Washington School of Medicine, Seattle, WA. Strain K12 was from the Food Technology Department culture collection. All strains were maintained on 2% peptone agar slants sealed with wax and stored at room temperature in the dark.

#### Culture conditions

Bacterial strains grown overnight in Matrix Milk, a milk-based starter culture medium (Galloway-West Co., Fond du Lac, WI), were inoculated at the 1% level ( $10^5$ - $10^6$  cells/ml) into fresh Matrix Milk adjusted to the proper pH and were incubated with shaking at 37 C for 24 h. These cultural conditions support excellent enterotoxin production by cultures grown in the Casamino acids-yeast extract (CAYE) medium of Evans et al. (3). Culture filtrates were obtained as previously described (6) and were adjusted to pH 7 before being tested

for enterotoxin activity.

#### Enterotoxin assays

The suckling mouse assay of Dean et al. (1) was used to test culture filtrates for ST activity as previously described (6). The average gut weight: body weight ratio of three mice was used for each culture. A ratio > 0.085 was considered a positive response.

The adrenal cell assay of Donta et al. (2) was used to test for LT activity, as previously described (6). Duplicate tests in two trials were used for each culture. Rounding of > 50% of the adrenal cells was considered a positive response to LT. Negative control wells contained 10-20% rounded cells.

#### Enumeration of organisms

Colony counts of Violet Red Bile Agar (Difco) were obtained for all cultures by using standard procedures (7).

### RESULTS AND DISCUSSION

The results of this study are presented in Table 1. Heat-labile enterotoxin activity was detected in the culture filtrate of strain 263 at all the initial pH values tested. None of the other strains produced detectable levels of enterotoxin under any condition tested. All the enterotoxigenic strains produced high levels of the appropriate toxin when grown in CAYE medium adjusted to pH 8.5 except for strain 263. This strain is designated as an ST/LT producer, but we were never able to detect ST activity in any culture filtrate. It is possible that the plasmid bearing the gene(s) for ST production was lost from this strain during storage.

Strain 263 grew more vigorously in milk than did the other strains, as evidenced by higher final colony counts and lower final pH levels in all cultures of strain 263. It is possible that strains 711 and 1261 produced enterotoxin at levels below our limits of detection. Culture filtrates were not concentrated before testing.

Although it is possible that enterotoxin activity would have been detected in the culture filtrates of the other strains if they had reached higher cell densities, it also is possible that enterotoxin synthesis is associated only with vigorous growth or that the proper nutritional conditions for enterotoxin production are lacking in milk. Strain 263 also may possess some unique property that enables it to produce enterotoxin in milk. In the only other reported attempt to produce enterotoxin in milk, the three enterotoxigenic strains used failed to produce detectable levels of LT or ST when grown at 37 C (J.

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TABLE 1. Growth and enterotoxin production in milk cultures by selected strains of *Escherichia coli*, incubated at 37 C for 24 h.

| Strain | Enterotoxin produced | Initial pH | Final pH | Final bacterial count <sup>a</sup> | Enterotoxin production measured in milk |                 |
|--------|----------------------|------------|----------|------------------------------------|-----------------------------------------|-----------------|
|        |                      |            |          |                                    | LT <sup>b</sup>                         | ST <sup>c</sup> |
| 263    | LT <sup>d</sup>      | 6.5        | 5.4      | 9.20                               | ± <sup>e</sup>                          | NA <sup>f</sup> |
|        |                      | 7.0        | 5.6      | 9.11                               | +                                       | NA              |
|        |                      | 7.5        | 5.5      | 9.10                               | +                                       | NA              |
|        |                      | 8.0        | 5.7      | 9.09                               | +                                       | NA              |
|        |                      | 8.5        | 5.7      | 9.18                               | +                                       | NA              |
| 711    | LT                   | 6.5        | 6.5      | 7.93                               | —                                       | NA              |
|        |                      | 7.0        | 6.8      | 7.85                               | —                                       | NA              |
|        |                      | 7.5        | 7.1      | 7.99                               | —                                       | NA              |
|        |                      | 8.0        | 7.4      | 7.84                               | —                                       | NA              |
|        |                      | 8.5        | 7.5      | 7.62                               | —                                       | NA              |
| 1261   | ST                   | 6.5        | 6.4      | 7.84                               | NA                                      | —               |
|        |                      | 7.0        | 6.7      | 7.94                               | NA                                      | —               |
|        |                      | 7.5        | 7.1      | 7.86                               | NA                                      | —               |
|        |                      | 8.0        | 7.3      | 7.89                               | NA                                      | —               |
|        |                      | 8.5        | 7.5      | 7.92                               | NA                                      | —               |
| K12    | None                 | 6.5        | 6.5      | 7.87                               | —                                       | —               |
|        |                      | 7.0        | 6.8      | 7.91                               | —                                       | —               |
|        |                      | 7.5        | 7.1      | 7.91                               | —                                       | —               |
|        |                      | 8.0        | 7.4      | 7.95                               | —                                       | —               |
|        |                      | 8.5        | 7.6      | 7.87                               | —                                       | —               |

<sup>a</sup>Log<sub>10</sub> colony forming units/ml, average of duplicate counts.  
<sup>b</sup>Results of two separate trials, duplicate test wells in each trial.  
<sup>c</sup>Average gut weight: body weight ratio of three mice.  
<sup>d</sup>This strain is designated as an LT/ST producer, but in our hands produced only LT.  
<sup>e</sup>Approximately 50% rounding of adrenal cells.  
<sup>f</sup>No assay performed.

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The initial pH of the medium also has been shown to be significant. Optimum enterotoxin production occurs at relatively high initial pH levels in laboratory media (5,11). In the current study, enterotoxin activity was marginal in the culture with an initial pH of 6.5, but was stronger in cultures at higher pH levels. The fact that the final pH values in all these cultures were approximately the same suggests that enterotoxin synthesis occurs until the pH of the medium falls below some critical value. Enterotoxin activity evidently is not inhibited by the low final pH of the culture.

It is unlikely that enterotoxin would be produced in fermented dairy products in which the pH fell below 6.5 relatively rapidly. Milk and other dairy products at a neutral pH also should be free of enterotoxin as long as they are stored under conditions which limit growth of *E. coli*.

**CONCLUSIONS**

Enterotoxin production by *E. coli* growing in milk is possible. Strain-to-strain variation may occur, with only very vigorously growing strains having the capability of producing significant amounts of enterotoxin. Normal storage conditions for dairy products should control *E. coli* growth and enterotoxin production.

**ACKNOWLEDGMENTS**

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# Food Allergy — The Enigma and Some Potential Solutions

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## ABSTRACT

Food allergy is one of the most common illnesses associated with ingestion of food. However, food allergy is often a loosely defined clinical phenomenon. An appeal is made for a stricter definition and more thorough diagnosis of food allergy. Food allergy should be a term reserved to describe those hypersensitive reactions to foods that have a demonstrated immunological basis. The incidence, symptoms and diagnosis of food allergy are discussed with some emphasis placed on subjects of current and continuing controversy. Several goals for future research programs in food allergy are stated, including (a) improvement of the food allergen extracts used in clinical diagnostic procedures, (b) development of adequate clinical procedures for diagnosis of delayed hypersensitivity reactions to foods and (c) development of methods for hypoallergenic processing of foods.

Food allergy remains an enigma within the area of food toxicology. Whereas many other aspects of food toxicology are drawing considerable research activity, food allergy continues to struggle for scientific attention. The lack of research activity on food allergy is not likely due to any lack of interest among food scientists. If not allergic to certain foods themselves, most food scientists are at least aware of the existence and importance of allergic reactions to foods through contact with allergic individuals, possibly even including their own families and friends. In addition, most food companies receive periodic complaints from consumers alleging allergic reactions to certain foods.

Multiple reasons exist for the dearth of research activities in food allergy. One major stumbling block that has limited progress and research in the field of food allergy is the lack of convenient and reliable methods for determining the allergenic activity of foods and food components. Usually a physician specializing in allergy must be consulted to perform such tests, since most of the available tests require use of consenting allergic patients. However, even with the assistance of a physician and his patients, the results of currently available tests are often not totally reliable. This unfortunate set of circumstances has almost certainly discouraged many food scientists from embarking on research programs in food allergy. In addition, many food scientists receive only minimal training in food allergy and consequently have limited knowledge and capabilities in this area. Meanwhile, physician-allergists have generally disdained food allergy because of their understandably meager knowledge of food chemistry and food processing and because of the greater incidence of allergies to pollens, molds, insect stings, etc. which has sparked research on

those allergies. Consequently, these factors, along with the generally held attitude that not much can be done to eliminate food allergy anyway, have created an effective barrier to research in food allergy.

This review will attempt to remove some of the enigma associated with food allergy by (a) carefully defining and classifying food allergies, (b) discussing the types of food allergy and their incidence, (c) briefly describing the symptomatology and clinical diagnosis of food allergy and (d) attempting to pinpoint some of the needs for future research in food allergy. Since it is impossible to discuss all relevant material, the reader is directed to several recent reviews for further information on clinical (32,33,52), chemical (1,10,45,58), immunological (17,42,44,58) and general (15,16,27,32,33,52) aspects of allergy and food allergy in particular.

## A MORE PRECISE DEFINITION AND CLASSIFICATION

Even the definition of the term, food allergy, is fraught with difficulty. Food allergy is often loosely defined as any unpleasant, abnormal or heightened response of an individual to a food or food component. However, this broad definition would lead to the classification of Chinese Restaurant syndrome and those forms of milk intolerance caused by a genetic deficiency of the enzyme, lactase, as food allergies. Obviously these two syndromes are quite different from each other and also distinct from the classical types of food allergy, e.g. as manifested by hives following ingestion of a given food by a sensitive individual.

A more precise definition of food allergy can be achieved. Many allergists prefer to limit use of the term allergy to those types of hypersensitivity that have a proven immunological basis (10,27,32,33). Chinese Restaurant syndrome and lactase deficiency-induced milk intolerance have no known immunological basis and would not be classified as food allergies under this more precise definition. Unfortunately, techniques necessary to demonstrate an immunological basis for a food-induced reaction are neither readily available nor entirely foolproof. Many clinical allergists do not have direct access to some of the most reliable techniques. For certain types of food allergy, particularly delayed hypersensitivity, adequate tests remain to be developed. Finally, even the best methods are not totally accurate. Consequently, even if the more precise definition of food allergy is widely adopted, some confusion will continue

until better methods become readily available. However, a good deal of the confusion surrounding food allergy could likely be eliminated if clinical allergists would uniformly apply the best of the available tests in the diagnosis of food allergy.

Adoption of the more precise definition of food allergy would lead to a better classification of the host of different conditions that comprise the unpleasant, abnormal and heightened responses of certain individuals to foods. Allergy would be reserved to describe those conditions with a proven immunological basis. Intolerance could be used to describe those conditions resulting from deficiencies or abnormalities in the enzymatic and/or biochemical mechanisms for metabolizing a normally non-toxic food component. Those conditions involving responses to a toxic chemical in a food would be classified as intoxications. Infection would be the classification for those conditions initiated by foodborne bacteria, viruses, rickettsia and parasites. Other conditions that defy classification in one of the other categories would simply be termed food sensitivities or idiosyncratic reactions.

Some allergists will undoubtedly argue with my definition of food allergy and classification of adverse reactions to food. Certain investigators have claimed that food allergy can be non-immunologic in its mechanisms, and thus not be recognized by the frequently used tests (15,16,19,20,59,64,67). According to this theory, individuals can have "masked" allergies and may be able to consume a certain food to which they are allergic without the occurrence of a clinically demonstrable immunological reaction. These contrary views seem to be supported mostly by testimonial case reports and appear to require further rigorous proof. Some scientists would prefer to include malabsorption diseases, inflammatory responses and enzyme deficiencies among the various forms of delayed or "occult" allergy (15,16).

#### TYPES AND INCIDENCE OF FOOD ALLERGY

Even if one decides to limit the definition of food allergy to conditions with a proven immunological basis, several distinct types of food allergy are known to exist. Often the types of food allergy are classified as immediate or delayed on the basis of the time between consumption of the food and the onset of adverse reactions (10,33). A more precise system of classification based on the type of immunological phenomenon observed has been described by Gell and Coombs (31) and has been widely adopted by other allergists (32,33,58). In this system, allergic reactions are grouped into four general types designated Types I through IV.

Immediate hypersensitivity-type allergic reactions appear to belong entirely within the Type I category. Type I reactions are often termed immediate hypersensitivity, reaginic hypersensitivity, anaphylactic hypersensitivity and atopy. Type I reactions are mediated by reaction of an allergen with a distinct type of immunoglobulin designated as IgE (some recent

information has implicated IgG<sub>4</sub> and IgD also) or sometimes by the older term, reagin. The molecular basis of IgE-mediated allergic reactions is fairly well understood and has been recently reviewed elsewhere (1,44). The observed symptoms of Type I hypersensitivity result from the release of pharmacologically active substances including histamine, serotonin, and others from mast cells as a consequence of IgE mediation. Type I reactions usually occur within several minutes to several hours following ingestion of the food.

Delayed hypersensitivity-type allergic reactions usually refer to the Type IV category (16,33). Type II and Type III reactions also exist (16,33), but have never been definitely documented in food allergies. Type II hypersensitivity reactions are produced by reaction of antibody with cell-bound antigen (allergen), followed secondarily by complement fixation. Examples of Type II reactions would be transfusion reaction and certain auto-immune diseases. Type III hypersensitivity reactions are caused by complement fixation which follows reaction and deposition of antigen-antibody complexes at some reaction site. An example of a Type III reaction would be serum sickness. Some forms of cow's milk allergy may be similar to Type III reactions but further proof is needed (38). Type IV hypersensitivity reactions, also termed delayed or cellular hypersensitivity, are created by the reaction of certain sensitized cells usually lymphocytes with allergen. Type IV reactions may occur with foods. The molecular basis of Type IV reactions is poorly understood by comparison to Type I reactions. The ultimate effect of Type IV reactions is a cytotoxic or cell destruction phenomenon. Type IV reactions can involve small molecular weight chemicals, including some food additives (49,69) that apparently act like haptens and react with tissue proteins before exerting their effects (53). The onset of symptoms in Type IV reactions typically occurs 6-24 h following ingestion of food.

May and Bock (52) have argued convincingly that the separation of food allergies into immediate and delayed categories is arbitrary. Their argument centers on the realization that the interaction of the allergen with IgE or sensitized cells is surely immediate in all immune reactions. Consequently, the interval between ingestion and the onset of symptoms is likely to be affected by the quantity of food consumed, the degree of hypersensitivity, successive and concomitant exposures, threshold for complaints and other factors (52). All of these factors are unrelated to the type of immunological phenomenon (IgE-mediated or sensitized cell-mediated) taking place. Consequently, differential categorization of food allergies as Type I or Type IV reactions must be based on demonstrated immunological differences and not simply on the time between ingestion and onset of symptoms.

Since allergists cannot even agree on a definition for food allergy, accurate estimates of the incidence of food allergy are virtually impossible to obtain. Other reviewers have reported a wide range of incidence figures for food allergy including 0.3% (33), 20% (4), 20% (6), 25% (15)

and 60% (71) of the population. It is generally agreed that not more than 1% of the population suffers from immediate hypersensitivity (Type I reactions). The argument concerns delayed hypersensitivity (Type IV reactions). Breneman (15,16), who estimates that 25% of the population suffers from some type of allergic disease, concludes that 95% of these allergies fall into the delayed hypersensitivity category. However, he would include malabsorption diseases, inflammatory responses and enzyme deficiencies as forms of delayed hypersensitivity. This broad definition of the term food allergy would undoubtedly serve to inflate his incidence figures. At the opposite end of the spectrum, Golbert (33) estimates that the incidence of allergy among children is 0.3%. He discounts delayed hypersensitivity, which is difficult to demonstrate clinically, as extremely rare. Consequently, his figures rely almost entirely on the incidence of immediate hypersensitivity.

This reviewer will not even attempt to estimate the incidence of food allergy other than to concede that it probably lies somewhere between 0.3% and 25% of the population. Even determining the existence of allergic reactions to specific foods is difficult. One problem with such determinations is the number of testimonial reports of food allergy in the scientific literature. One example chosen randomly from many possible candidates follows. Rousseau (64) described the case of an adult female who experienced giddiness which was exacerbated by honey and grapefruit. Honey and grapefruit are not usually considered allergenic foods and giddiness is not a normally described allergic symptom. Rousseau (64) provided no immunological testing of this patient. Consequently, this case should be classified as food sensitivity but further investigation might reveal an allergic basis. This example should serve to illustrate the problem.

#### SYMPTOMS OF FOOD ALLERGY

A variety of symptoms, including cutaneous, gastrointestinal and respiratory manifestations, have been associated with Type I reactions. The most common cutaneous symptoms are urticaria (hives) and angioedema (swelling), although eczema or atopic dermatitis has also been described. Eczema may be a Type IV rather than a Type I symptom, although the literature is confusing on this point. The gastrointestinal symptoms frequently include vomiting, abdominal cramps, nausea and diarrhea. The occasional reports of steatorrhea (fatty fecal discharge), colic and stomatitis (inflammation of the mucous membranes of the mouth), and other gastrointestinal symptoms (8) are either rare or largely unsubstantiated (52). The respiratory symptoms include rhinitis (inflammation of the nasal membranes) and asthma most commonly, although respiratory symptoms tend to occur far more frequently in pollen allergies than with food allergies. Food-allergic individuals with Type I reactions usually experience no more than a few of these symptoms. Heiner's syndrome (8,38) should also be

mentioned here, although it may not be manifestation of Type I reactions. Heiner's syndrome was originally described in relation to cow's milk allergy in infants and consists of poor weight gain, gastrointestinal and upper respiratory symptoms, recurrent pulmonary disease and iron deficiency anemia (8,38). Heiner's syndrome involves antibodies other than IgE, and may be due to the accidental aspiration of milk (52).

The association of certain symptoms with food allergy has been somewhat controversial. The controversial symptoms have included common symptoms such as eczema or atopic dermatitis and asthma and uncommon symptoms such as otitis media and Meniere's disease. Several recent studies have been performed on the association between these symptoms and food allergy. In a study of 134 subjects with atopic dermatitis, Bonifazi et al. (14) noted that only 45 had a clinical history of food sensitivity to eggs, milk or cod, while 79 showed clinical evidence of allergic sensitivity to these foods by virtue of the *in vitro* radioallergosorbent test (RAST). These workers concluded after monitoring IgE levels that atopic dermatitis was not necessarily attributable to specific sensitization by distinct food allergens, but was characterized by an excessive production of IgE (14). Similarly, Hammer (37) demonstrated that only 15 of 81 children less than 5 years old with atopic dermatitis suffered symptom exacerbation on oral provocation with cow's milk and/or cereals (wheat, rye and oat mixture). In this study, initial serum IgE levels and evidence of specific antibodies by RAST did not distinguish a sensitive group. On the other side of the controversy, 14 of 20 infants with atopic dermatitis showed improvement on a diet eliminating cow's milk, eggs, chicken and beef (7). High-risk infants with an allergic parent showed a lessened incidence of eczema if breast feeding was maintained for the first 12 weeks of life and milk products, eggs and fish were avoided for the first year (3,50). Some recent immunologic findings suggest that food-allergic persons with atopic dermatitis also have defective immune surveillance systems, including defective cell-mediated immunity, decreased T lymphocyte numbers and defective effector cell functions (26), all of which could lead to increased IgE levels and food allergen sensitivity. With asthma, food allergy seems to play a role in a consistent, though small, percentage of the affected individuals. A recent study with 147 asthmatic children and 250 asthmatic adults indicated that 15% of the children and 17% of the adults had food allergies, as demonstrated clinically by the skin test (29). Secretory otitis media and Meniere's disease are rarely reported symptoms of food allergy. Estimates have been made that from 5 to 25% of the cases of secretory otitis media have an allergic component (62,63). Some of these individuals respond to elimination of certain foods from their diets (62), although more careful studies are needed to substantiate this relationship. The involvement of food allergy in Meniere's disease is uncertain. Holloman (39) has discounted the importance of food allergy in



Meniere's disease, but occasional reports continue to surface that implicate foods in the etiology of this condition (23).

A particularly serious manifestation of Type I hypersensitivity is anaphylaxis. Anaphylaxis is a severe, generalized shock reaction that can result in death. Fortunately, anaphylactic reactions to foods are rarely reported. Within the past year, anaphylactic reactions to cow's milk (60), sunflower seeds (55), shellfish (51) and gum tragacanth (21) have been reported. Severe systemic reactions are known to occur occasionally with the more frequently reported allergenic foods such as peanuts, fish and egg whites.

The manifestations of food-associated Type IV reactions are less clearly defined. Contact dermatitis associated with the handling of certain foods and food components by sensitive individuals has been demonstrated (9,49,65). Gastrointestinal, respiratory and other symptoms of allergy are difficult to confirm in Type IV reactions. Some allergists believe that Type IV reactions to foods are rare (33), while others would consider them to be common (15,16,19). Numerous symptoms have been ascribed to delayed hypersensitivity reactions but the evidence in many cases is sketchy. The possible association of certain behavioral disorders with Type IV reactions has been the subject of continuing conjecture. Speer (67) originally coined the term, allergic tension-fatigue syndrome, to describe a rather complex set of behavioral changes that he noted in certain allergic individuals. The fatigue symptoms included motor fatigue (tiredness and achiness), sensory fatigue (sluggishness, torpor) and mental fatigue (depression, paranoia etc.). The tension symptoms included motor tension resembling hyperkinesia and sensory tension (irritability, insomnia, etc.). Widespread support for the idea of a direct correlation between allergy and behavioral symptoms has never surfaced. Recently, Crook and others (19,20,59,62) have suggested that these behavioral symptoms may be manifestations of Type IV reactions to foods, although their reports have been largely testimonial. The suggestion has been made that hyperkinesia may have an allergic basis (19). One report links symptom exacerbation in an autistic child with certain foods (56). Recent studies have demonstrated that a small percentage of children with various behavioral disorders have food allergies and/or revert some symptoms upon withdrawal of certain foods (41,70). Further support for an association between hyperkinesia and allergies comes from preliminary findings that suggested that cromolyn DSG, a drug that blocks histamine release from mast cells, had a positive behavioral effect on allergic hyperkinetic and autistic children (65). Considerable research is obviously needed to document further the manifestations of Type IV reactions to foods.

Numerous unusual symptoms have also been ascribed to food allergy. Since many of these symptoms were reported in testimonial style with no immunological

evidence of allergy, they would mostly fall into the category of food sensitivity. That vast number of such reports eliminates the possibility of any thorough cataloguing of the symptoms or the suspect foods. Several recent reports have included anxiety, depression, agoraphobia, headache, aphthous ulcers, localized pain, chronic nausea, lethargy, giddiness, sweating, food craving, itch, general weakness, heatwaves, palpitations, blurred vision and a feeling of suffocation as symptoms of food allergy (24,54,64). The multiplicity of symptoms and a lack of general agreement among clinicians regarding which symptoms to definitely associate with allergic reactions have contributed significantly to the problems associated with correctly diagnosing food allergy.

#### CLINICAL DIAGNOSIS OF FOOD ALLERGY

Since this review is not intended to be clinically oriented, only limited comments on the clinical diagnosis of food allergy will be made. The reader is directed to several recent reviews for more complete discussions of this topic (32,33,52).

The diagnosis of food allergy remains a controversial subject. Numerous methods have been developed for the diagnosis of immediate hypersensitivity to foods, including subjective methods (clinical histories, diet diaries, and elimination-challenge studies), the more objective double-blind challenge studies, various cutaneous tests (intracutaneous test, direct skin test, and Prausnitz-Küstner or passive transfer test), and indirect, *in vitro* testing methods (pulse acceleration, leucopenic index, urinary protease, eosinophilia, serum immunoglobulin levels, radioallergosorbent tests, cytotoxic reactions, leukocyte histamine release, lymphocyte transformation tests and intestinal biopsy). The pulse acceleration test, leucopenic index and urinary protease test have fallen into general disfavor due to lack of accuracy. The other tests continue to be used to some degree in the diagnosis of Type I reactions to foods. Methods for the diagnosis of delayed hypersensitivity to foods are not generally available. Cutaneous Type IV reactions such as contact dermatitis can usually be confirmed by patch testing (58,66). Diagnosis of other manifestations of Type IV reactions usually relies on some type of elimination-challenge study. Since these responses are subjective, the methods are not wholly reliable.

The most readily available diagnostic procedures for food allergies for both Type I and Type IV reactions are the subjective methods of history-taking, elimination diets and challenge studies. Johnstone (43) suggests that despite the recent proliferation of indirect methods for diagnosis of Type I reactions, no marked improvement has been made on the use of careful histories and elimination diets. One recent study showed that food-sensitive patients remitted 70% of their allergic symptoms when placed on a chemically defined diet (40). Subsequent oral challenge studies allowed identification of the offending foods (40). The chemically defined diet was an elemental diet containing L-amino acids, glucose,

glucose oligosaccharides, and safflower oil (28). This diet caused remission of symptoms in 85% of the food-sensitive patients studied (40). The use of double-blind challenge studies in the diagnosis of food allergy has been recommended (32,52). Double-blind challenges may be extremely useful in the confirmation of clinical histories. May and Bock (52), using double-blind studies of children with positive histories, were able to provoke symptoms with foods in only 33% of children 3 to 16 years of age and 52% of children less than 3 years of age. The employment of a double-blind design in food challenge studies should improve the objectivity of such evaluations. Certainly in cases where immunological evaluation is not available, such as with certain suspected cases of delayed hypersensitivity, the use of double-blind challenge tests is the preferred procedure.

While the use of such subjective tests is considered by some allergists to be reasonably definitive in the diagnosis of food allergy, others have stressed the necessity of determining an immunological basis for food allergy (10,27,32,33). The most commonly used tests to demonstrate such an immunological basis are the skin test, the radioallergosorbent test (RAST), histamine release from leukocytes and the passive transfer test. These tests are limited mostly to the diagnosis of Type I reactions. The direct skin test involves the intracutaneous introduction of the food extract and observation for an immediate wheal-and-flare reaction (13,33). The RAST is an *in vitro* test using a small amount of patient's serum. The RAST is available only in certain laboratories due to the need for some specialized equipment and reagents. The RAST has been described in detail by Yunginger (73). The other tests have been performed on a more limited basis for a variety of reasons relating to difficulty, risk to the patient and comparative accuracy.

Unfortunately, the results of the skin test and the RAST do not always agree even though both tests are mediated by IgE (2,18,30,57). In addition, neither the skin test nor the RAST always agree with the clinical histories of the patients (2,13,14,37,52). The agreement is better for certain foods than for others (2). Several reasons may exist for the occasional disparate results between the tests and the clinical histories. As mentioned previously, clinical histories are not invariably accurate although the accuracy can be improved by double-blind challenge studies (52). While the skin tests and RASTs are both mediated by IgE, some technical differences exist. May and Bock (52) suggest that the RAST is less reliable because it depends on serum IgE, and IgE has a short half-life in the circulation. IgE fixed in the skin has a longer half-life, making the skin test somewhat more stable as an index of allergenicity (52). However, the RAST has been used effectively in numerous diagnostic laboratories (1,73). The skin test is not perfect either since it relies on the skin response to released mediators primarily histamine. The histamine responsiveness of the skin may vary between individuals and is not perfectly

correlated with serum IgE levels. Other factors may shed some doubt on the reliability of either the skin test or the RAST in the diagnosis of food allergy. Both the skin tests and the RASTs require food extracts containing the allergen. The extracts used in clinical situations are usually rather crude preparations that can be obtained commercially. The standard practice seems to be to produce these food allergen extracts from fresh unprocessed foods. While the use of fresh material may have some validity with pollen allergens, it has dubious value in preparing food allergen extracts. For example, why prepare a peanut allergen extract from raw peanuts when the vast majority of consumers eat roasted peanuts, peanut butter or other processed forms of peanuts? This practice may represent convenience rather than logic. Some food allergens, such as the coffee bean allergen (47), are known to be destroyed by processing. The effect of processing on the allergenicity of most foods has not been investigated. However, use of unprocessed food extracts may lead to misdiagnosis of food allergy and a disparity between the skin tests or RASTs and the clinical histories. Fries (25) suggests that such misdiagnosis may occur rather frequently with allergy to chocolate.

Another factor adding to the difficulty in the diagnosis of food allergy is the lack of purified standards to use in the extracts. The presence of other substances in the crude extracts almost certainly confounds the diagnosis of food allergy on occasion by either inhibiting or promoting a positive response. Only a few food allergens have been isolated and identified. The most notable effort has been the purification of allergen M from cod (5,22).  $\beta$ -Lactoglobulin appears to be the most active milk allergen (11,34,35,46), although reactions to casein,  $\alpha$ -lactalbumin, and bovine serum albumin have also been observed (46). It should be noted that  $\beta$ -lactoglobulin and casein are stable to heat in excess of 100 C, while  $\alpha$ -lactalbumin and bovine serum albumin are heat-labile (48). The most active allergen in egg whites is the heat-stable protein, ovomucoid (12,72). Reactions to ovalbumin and lysozyme have also been noted to a lesser extent, while conalbumin seems to be non-allergenic (72). The association of heat-stable proteins with allergenicity undoubtedly allows them to survive certain types of processing.

A further complicating factor in the diagnosis of food allergy is the effect of proteolytic digestion on the allergenicity of food proteins. Many food allergens are stable to proteolytic digestion (1,10) but some notable exceptions may exist. Spies et al. (68) demonstrated that with cow's milk proteins, pepsin hydrolysates were more antigenic than the native proteins. Haddad et al. (36) recently confirmed this finding by showing that while only four of 10 milk-allergic patients had positive RASTs to  $\beta$ -lactoglobulin, 10 of 10 had positive RASTs to pepsin-trypsin digests of  $\beta$ -lactoglobulin. This result suggests that the clinical diagnosis of cow's milk allergy could be improved by employing proteolytic digests in

the allergenicity tests. More research is needed to determine the allergenicity of proteolytic hydrolysates of other allergenic food proteins.

#### FUTURE RESEARCH NEEDS IN FOOD ALLERGY

Three areas of research on food allergy need to be investigated to provide some solutions to the continuing enigma surrounding food allergies.

(a) A need exists to improve the quality and reliability of the food allergen standards used in clinical diagnostic procedure. The ultimate reliability would be achieved with a series of highly purified food allergen preparations. However, purification of the numerous food allergens will be time-consuming work and the eventual cost may be prohibitive. Some increase in reliability might be obtained by simply preparing the crude extracts from processed foods.

(b) To substantiate the claims of Crook (19,20), Breneman (15,16) and others that delayed hypersensitivity or Type IV reactions to food occur with reasonably high frequency, better methods for diagnosis of non-cutaneous Type IV reactions are needed. A better understanding of the molecular mechanisms of delayed hypersensitivity may be prerequisite to the development of such methods.

(c) To completely destroy the old myth that nothing can be done about food allergy anyway, let me emphasize that the food industry is already performing some positive preventive measures. For example, green coffee beans are quite allergenic (47). Consequently, roasting serves as a hypoallergenic process (47). Substitution of soybean-based and other formulas for cow's milk has been another positive step in the formulation of hypoallergenic foods. Considerable research is needed on the allergenicity of various foods. Perhaps methods can be developed for the hypoallergenic processing of various foods.

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### Gaspak, *con't from p. 291*

pound for canned or frozen products.

The energy savings are based on a number of factors that distinguish "Gaspak" from canning and freezing. In transportation, for instance, there is no need to refrigerate "Gaspak" produce. In a similar vein, "Gaspak" products are not packed in water, as are those that are canned, where water constitutes as

much as 40 percent of some can contents.

Frozen foods require the most energy in home cooking. Energy used in cooking "Gaspak" food is comparable to that used in cooking pre-thawed frozen foods.

Dr. Kramer says that if the "Gaspak" technology replaced only the canning and freezing of fruits

and vegetables, the estimated energy savings would be the equivalent of about 25 million barrels of crude oil. The estimated energy savings of industrial plants now processing

these foods would be 75 to 80 percent and reduced energy means reduced food processing costs, Dr. Kramer notes.

## Status of *Salmonella* — Ten Years Later

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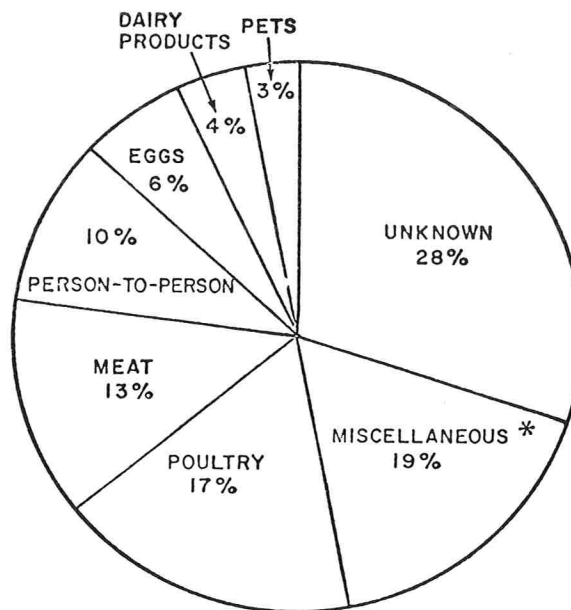
### ABSTRACT

The current status of the *Salmonella* problem, 10 years after publication of the report of the Committee on *Salmonella* of the National Research Council, was reviewed. The incidence of human salmonellosis has increased during this period. The major source of the *Salmonella* problem in man still derives from foods of animal origin, especially poultry, beef and pork. Contaminated products cause disease as the result of inadequate cooking or cross-contamination of working surfaces in the kitchen environment. The epidemiology of human salmonellosis has not changed during the past decade. Surveillance remains a powerful tool for protecting the consumer by discovering on-going epidemics, but it has done little to control the endemic problem from which epidemics emerge. Several outbreaks during the past decade indicate that the infecting dose, at least for certain *Salmonella* strains in certain foods, is considerably lower than that indicated on the basis of volunteer studies with adult male prisoners. The National Research Council report, as well as those from numerous other groups, emphasized the need for consumer education if the incidence of food-associated outbreaks is to be reduced. The rising incidence of human salmonellosis indicates that consumer education programs have not been successful. It was suggested that courses of study in proper food handling practices could profitably be directed toward students in secondary schools and that as a result, on a long term basis, the incidence of foodborne illness, including salmonellosis, would be reduced. Since a large percentage of outbreaks is traced to mishandling of foods in commercial establishments, it was suggested that inspectional activities in hotels, restaurant and catering facilities be increased at the expense of decreased activities in food processing facilities.

It has been ten years since publication of the report prepared by the Committee on *Salmonella* of the National Research Council (27). It is the purpose of this paper to review the decade since the issuance of that report.

Poultry, meat, eggs and dairy products are still the most important vehicles of transmission, as indicated in Fig. 1 (20). The proportion of outbreaks caused by eggs has declined dramatically as control measures have been instituted. Clearly, the major source of the *Salmonella* problem in man derives from foods of animal origin, especially poultry, beef and pork. Infection of animals occurs on the farm, and infected animals transmit the disease to uninfected animals during transit to processing plants or while being held before slaughter.

The contaminated products cause disease as a result of inadequate cooking or cross-contamination of working surfaces in the kitchen environment. Large outbreaks are almost always associated with foods prepared in foodservice establishments, such as hotels, restaurants and institutions and by catered foods. Various food



\* INCLUDES OVER 50 VEHICLES WHICH INDIVIDUALLY CAUSED LESS THAN 3% OF OUTBREAKS

Figure 1. Mode of transmission in 500 human salmonellosis outbreaks, 1966-1975 (4).

handling errors are woven into almost all studied outbreaks. This is evident from the data in Table 1 (11).

Obviously, these facets of the human salmonellosis problem are the same as existed 10 or even 20 years ago. The statistics are essentially the same, the foods involved have not changed and the same handling errors continue.

In 1967, the FDA inaugurated intensive efforts to eliminate salmonellae from the U.S. food supply. In its Task Force Report in 1973 (1), the FDA concluded that its efforts over the years 1966-71 had been effective in controlling the overall incidence of foodborne salmonellosis caused by foods other than meats. This claim was based upon the data in Table 2. The report further stated: "Since the inception of intensified FDA efforts in 1967, the incidence of foodborne salmonellosis attributable to foods other than meat has dropped to 25% of the reported outbreaks, while the estimated total number of human isolations has remained relatively constant." The report concludes: "This trend suggests a 25-35% reduction in the incidence of salmonellosis caused by foods except meat since the base years." Tompkin (30) has taken issue with this conclusion, pointing out that

TABLE 1. Factors that contributed to the occurrence of 61 outbreaks of foodborne salmonellosis, 1973-1976.

| Contributing factors                                      | Number           |            |                                  | Total (61) | Percent |
|-----------------------------------------------------------|------------------|------------|----------------------------------|------------|---------|
|                                                           | Foodservice (28) | Homes (20) | Food Processing and markets (13) |            |         |
| <i>Factors affecting growth</i>                           |                  |            |                                  |            |         |
| Inadequate cooling                                        | 20               | 6          |                                  | 26         | 43      |
| Preparing foods a day or more before service <sup>1</sup> | 7                |            |                                  | 13         | 16      |
| Inadequate hot storage                                    | 10               | 1          |                                  | 11         | 13      |
| Use of leftovers <sup>2</sup>                             | 3                |            |                                  | 3          | 5       |
| Faulty fermentations                                      |                  |            | 2                                | 2          | 3       |
| <i>Process failure</i>                                    |                  |            |                                  |            |         |
| Inadequate reheating                                      | 14               |            |                                  | 14         | 23      |
| Inadequate cooking                                        | 1                | 3          | 3                                | 7          | 11      |
| <i>Factors affecting contamination</i>                    |                  |            |                                  |            |         |
| Contaminated raw ingredients                              | 2                | 11         | 7                                | 20         | 33      |
| Cross-contamination <sup>2</sup>                          | 9                | 3          |                                  | 12         | 20      |
| Inadequate cleaning                                       | 9                | 1          | 1                                | 11         | 18      |
| Infected persons <sup>3</sup>                             | 6                | 1          | 1                                | 8          | 13      |

<sup>1</sup>Indirect factor.

<sup>2</sup>Data not available for contamination from incoming animals to be processed or raw foods of animal origin.

<sup>3</sup>Unknown whether or not infected persons were initial sources of organism or ate implicated foods.

From: Bryan (11).

TABLE 2. Incidence of outbreaks of human salmonellosis attributable to meat and meat products as opposed to other foods.<sup>a</sup>

| Product                | 1963-66 |         | 1967-71 |         |
|------------------------|---------|---------|---------|---------|
|                        | Number  | Percent | Number  | Percent |
| Meat and meat products | 27      | 63      | 53      | 75      |
| Other foods            | 16      | 37      | 18      | 25      |
| Total                  | 43      |         | 71      |         |

<sup>a</sup>Source: FDA *Salmonella* Task Force Report (1).

the number of reported outbreaks involving meat and poultry products fluctuated upward during the period 1967-1971, whereas the number of outbreaks attributed to other foods remained steady and certainly did not decline. Tompkin's analysis of the data appears to be correct.

In 1967 the FDA concentrated almost half its sample analysis efforts on five product categories (eggs, dry milk, candy, drug substances of animal origin and miscellaneous foods, such as starches and sugars). Eight and six tenths percent (8.6%) of these samples were positive. In FY 1972 only 0.8% of these samples were positive, certainly indicating improvement. The data in Table 3 suggests that the incidence of contamination for other processed human foods did not change materially during the period from 1967 to 1972 (1).

Nonfat dry milk is under the USDA *Salmonella* Surveillance Program. Data in Table 4 indicate that in the 10-year period from 1967 to 1978 there had not been a change in the incidence of positives detected in connection with this program (19). No doubt the continuing surveillance program is detecting problem plants and thus preventing the movement of contaminated product into the market place.

### SURVEILLANCE

Surveillance remains today, as 10-15 years ago, a powerful tool for protecting the consumer by discovering

TABLE 3. Results of samples of food and feed products analyzed by FDA between the years FY 1967 and FY 1972<sup>a</sup>.

| Categories                                     | FY 1967                 |                  | FY 1972                 |                  |
|------------------------------------------------|-------------------------|------------------|-------------------------|------------------|
|                                                | Number samples examined | No. (%) positive | Number samples examined | No. (%) positive |
| Top 5 product categories                       | 2849                    | 244 (8.6%)       | 368                     | 3 (0.8%)         |
| All other categories except animal by-products | 3239                    | 254 (4.0%)       | 1337                    | 70 (5.2%)        |

<sup>a</sup>Source: FDA *Salmonella* Task Force Report (1).

TABLE 4. USDA - *Salmonella* Surveillance Program Data for NDM<sup>a</sup>.

| Year | Number of product samples tested | % <i>Salmonella</i> positive |
|------|----------------------------------|------------------------------|
| 1967 | 7,843                            | 0.7                          |
| 1968 | 17,496                           | 0.2                          |
| 1969 | 12,822                           | 0.3                          |
| 1970 | 11,254                           | 0.36                         |
| 1971 | 25,321                           | 0.27                         |
| 1972 | 28,736                           | 0.17                         |
| 1973 | 16,652                           | 0.31                         |
| 1974 | 12,048                           | 0.52                         |
| 1975 | 10,423                           | 0.71                         |
| 1976 | 14,418                           | 1.9                          |
| 1977 | 16,517                           | 1.08                         |
| 1978 | 17,224                           | 0.60                         |

<sup>a</sup>From: Mr. George Fry, Dairy Grading Branch, Poultry-Dairy Quality Division, FSQS, USDA, Washington, D.C.

on-going epidemics. Numerous examples could be cited: *Salmonella newbrunswick* in powdered milk - 1972 (14), *Salmonella eastbourne* in chocolate - 1975 (15) and *Salmonella heidelberg* in Cheddar cheese - 1977 (13). In each of these cases, the offending product was removed from the market and the spread of disease checked.

One of the most notable triumphs of surveillance came from the discovery of an association between nontyphimurium salmonellosis in children and pet turtles. This finding led to enactment of regulations prohibiting the interstate shipment and importation of pet turtles. As a result of this, it is estimated the American public was

spared approximately 300,000 cases of salmonellosis each year. As Gangarosa (20) has pointed out, the benefits of these control measures can be directly quantitated in a reduction in attack rates caused by reptile-associated *Salmonella* serotypes, but the overall national morbidity has remained unchanged, because *Salmonella agona* emerged as one of the leading serotypes at the same time a reduction was occurring from the pet turtle regulation.

The situation with *S. agona* illustrates that we are still finding that when a new serotype is introduced into a geographical area, it may ultimately find its way into the human population (12). Before 1970, *S. agona* was reported in man only twice, once in 1967 and once in 1968. One of the first isolates in the United States was from a consignment of Peruvian fishmeal shipped to the southeastern United States in March 1970. Although importing countries had examined Peruvian fishmeal for many years, *S. agona* was first isolated in 1969. This suggests that contamination of Peruvian fishmeal was a discrete event that occurred in that year. After this initial isolation, *S. agona* was reported with increasing frequency from nonhuman sources. Several months after the increase in nonhuman *S. agona* isolations, the number of cases in man began to increase. In 1969-70, *S. agona* likewise emerged as a public health problem in the United Kingdom, Israel and the Netherlands (12). Figure 2 (20) dramatically illustrates the rise in importance of this serotype. In 1976, it ranked third

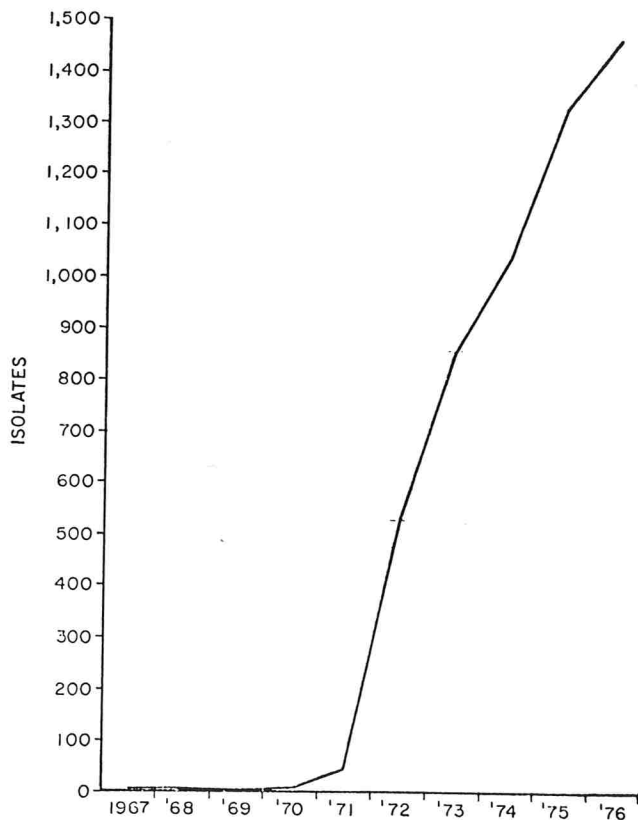


Figure 2. Isolates of *Salmonella agona* from human sources, United States, 1967-1975 (20).

among the then most frequently isolated serotypes from human sources and fourth from nonhuman sources.

It is clear that surveillance has accomplished a good deal to control epidemics, but it has done little to control the endemic problem from which epidemics emerge. Gangarosa (20) has referred to the surveillance effort as a firefighting operation, one which has not addressed itself to the root issue which is the vast reservoir in the animals man depends upon for his food. Indeed, neither red meat nor poultry products are seized or confiscated because of *Salmonella* contamination. This was true at the time the report of the *Salmonella* Committee was published, and it is true today. Regulatory policies are inconsistent, since *Salmonella*-contaminated red meat and poultry move in commerce, but contaminated imported frog legs and shrimp are detained. Certainly the risks are comparable, and justification for detention could be based only on the fear of introducing new serotypes into the domestic environment.

#### FEED AND FEED INGREDIENTS

The *Salmonella* Report (27) recommended that federal and state agencies develop and implement programs to control *Salmonella* contamination of feed and feed ingredients. Both the FDA and the USDA have administered programs to this end, but these programs have now been abandoned. In early 1976, the FDA indicated that it would not initiate any new federal regulatory programs directed at the animal and marine by-products industry, with the exception of those individual cases exhibiting a clear and present danger to public health (10). The decision was greatly influenced by a literature search and analysis conducted by E. M. Foster and R. H. Deibel. Their report emphasized that there are many potential sources of *Salmonella* infection in animals, that no single source can be clearly implicated as the major contributor to the spread of disease in animals. They concluded that any program to eliminate *Salmonella* from rendered animal and marine by-products would result in higher prices for the feed industry and ultimately for the consumer, that promulgation of any program would involve an unacceptably high expenditure of resources. The Center for Disease Control (CDC) does not agree with the FDA-USDA positions, holding that much human salmonellosis relates directly or indirectly to *Salmonella*-contaminated animal feeds. The CDC believes that a program to control *Salmonella* in animal feeds would contribute significantly to a reduction in human salmonellosis (2). At the moment the positions of contaminated red meats and poultry, contaminated animal feeds and contaminated rendered animal by-products are the same as existed when the *Salmonella* report was issued. The report of the joint FAO/WHO Expert Committee on Food Microbiology (8) contains recommendations that are consistent with the position of the FDA and USDA with respect to red meats and poultry.

### PRODUCT CLASSIFICATION

A novel feature of the *Salmonella* Committee Report was the suggestion of a system of food product classification on the basis of potential health hazard (27). This was based upon three hazard characteristics: (a) whether the product contains an ingredient that has been identified as a significant potential factor in salmonellosis, (b) whether the manufacturing process does not include a control step that would destroy salmonellae and (c) whether there is a substantial likelihood of microbiological growth if the product is mishandled or abused in distribution or consumer usage. Processed foods and food ingredients were arranged according to five different categories in relation to potential hazard. Category I was reserved for food products intended for use by infants, the aged and the infirmed. Categories II through V related to decreasing degrees of risk. Acceptance criteria for each product category were recommended.

The Committee's intent was to provide a way to clear suspect products. It was not intended that these sampling plans would be used for routine surveillance. Plans were devised because of the widely recognized need to decide what to do with a suspect lot of product. The FDA was acutely in need of a way to know when to quit testing. When the agency had reason to believe that salmonellae might be in a food product or ingredient they often tested over and over again ad absurdum. The NRC sampling plan gave them, and everybody else, a basis for decision. The FDA accepted the sampling plans for surveillance and/or compliance for regulatory consideration.

Though the extensive analysis of multiple 25-g samples, as prescribed in the NRC report, was extremely expensive, industry followed these procedures in evaluating suspect lots. Subsequently, research indicated that multiple 25-g samples could be pooled to provide large composite samples for analyses (29). Statistical quality control over the *Salmonella* defect then became economically feasible, and accordingly large segments of the food industry started to utilize the NRC sampling plans for quality control purposes. This continues to be the case.

The 1978 edition of *The Bacteriological Analytical Manual* (BAM) (18) re-defined food classifications, recognizing three categories. Aside from Category I, classification is based upon whether foods would normally be subjected to a process lethal to *Salmonella* between the time of sampling and consumption. Those which would not normally receive this treatment are placed in Category II. Those which would be placed in Category III. This change caused a number of interesting shifts in classification, as indicated in Table 5. It will be noted that a number of seemingly innocuous foods have been shifted from Category III to Category II. On the other hand, a number of potentially more dangerous foods are classified in Category III, within the scope of this definition.

TABLE 5. Selected examples of categories - BAM 1976 (17) vs. BAM 1978 (18).

| Foods                                                                                                                                              | BAM Classification |         |
|----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|---------|
|                                                                                                                                                    | 1976               | 1978    |
| Salt, flavors and extracts, mayonnaise, fresh fruits and juices, jams, soft drinks, water, beverage bases, coffee, tea, snack items (dry), syrups. | CAT. III           | CAT. II |
| Frozen dinners                                                                                                                                     | II                 | III     |
| Fresh and frozen shellfish and crustaceans (ex. raw oysters and clams), other aquatic animals, fresh vegetables.                                   | III                | III     |
| Sampling: Category II - 30 - 25-g samples<br>Category III - 15 - 25-g samples                                                                      |                    |         |

In general, the applications of these acceptance criteria have brought a modicum of order out of the chaos that was the "zero tolerance." But it should be mentioned that even with these plans and categorizations, the FDA has not always been consistent. In late 1977, one or more lots of imported brewer's yeast were found to contain *Salmonella*. Before this was discovered, the dried yeast had been incorporated into innumerable lots of health food tablets, vitamin pills and other concoctions distributed in health food stores. Working with the State of California, the manufacturers sampled suspect lots according to the Category I sampling scheme, and the samples were analyzed using BAM procedures. In Silliker Laboratories, we found 33 different lots of various products negative according to the Category I sampling scheme. The State of California was willing to permit these lots, and other lots cleared by other commercial laboratories, to be offered for sale. However, the FDA took the position that all of these lots had to be treated as adulterated, that they could not be offered for sale unless an approved decontamination procedure be applied. The FDA position in this episode was inconsistent with that which it has taken in a number of other instances and as a consequence worked unfair hardship on a number of manufacturers.

### INFECTIVE DOSE

Ten years ago little was known with regard to infecting dose. Our thinking was wrongly influenced by the work of McCullough and Eisele (24-26) which involved feeding studies conducted on healthy adult male prisoners. These results, summarized in Table 6, indicated dosage on the order of 1,000,000 was necessary to cause clinical illness. The disparity between infecting dosage and the zero tolerance made industry paranoid, feeling victimized by regulatory over-kill.

Study of a number of outbreaks in recent years indicates that the infecting dose, at least for certain strains in certain foods, is considerably lower than indicated in Table 6. Examples are listed in Table 7. Although the illnesses caused by carmine dye and pancreatin were in high-risk populations, the same can



not be said for the ice cream, chocolate candy and raw hamburger outbreaks. The unusually low numbers associated with the foods involved in these outbreaks suggest that the infecting dose may be linked to the chemical composition of the food, that the food may protect the organisms during passage through the stomach into the intestinal tract.

*S. agona* is listed in this table, because there is evidence that this organism is particularly virulent and is transmitted with relatively low infective doses (5). The organism also tends to acquire drug resistance by mutation or R-factor transfer. Incidentally, the *Salmonella newport* strain which caused the multi-state raw hamburger outbreak was resistant to three antibiotics commonly added to cattle feed, and this of course raises the question of the wisdom of feeding antibiotics that are frequently used in human medicine to livestock (16).

### THE FUTURE

As is usually true, an examination of the past gives the best clues of what the future may hold. If anything, the incidence of human salmonellosis is increasing - see Fig. 3 (4). Better reporting or interest bias can hardly account for the apparent failure of increased efforts to decrease the magnitude of the problem. The time span covered by the surveillance program is simply too great. As indicated at the outset, epidemiological patterns have not changed. Mishandling or misuse of raw foods is still the primary problem. The large well-publicized outbreaks are probably only maxi versions of the mini or home-based outbreaks that occur every day. Surveillance, primarily by CDC, has effectively detected and in many instances checked the larger outbreaks. Only rarely are the home episodes brought to the attention of health authorities and properly diagnosed. Therein lies the base of the much maligned *Salmonella* iceberg.

TABLE 6. Influence of serotype and infecting dose on clinical disease<sup>a</sup>.

| Type                  | Infecting dose (Millions) | Volunteers     |                  |                        |            | Days of excretion |        |                       |        |
|-----------------------|---------------------------|----------------|------------------|------------------------|------------|-------------------|--------|-----------------------|--------|
|                       |                           | Number exposed | Clinical illness | Symptom-less excretion | No. effect | Clinical illness  |        | Symptomless excretion |        |
|                       |                           |                |                  |                        |            | Range             | Median | Range                 | Median |
| <i>S. meleagridis</i> | - 0.16                    | 24             | —                | 13                     | 11         | —                 | —      | 2 - 21                | 4      |
|                       | - 5.50                    | 53             | —                | 44                     | 9          | —                 | —      | 2 - 128               | 9      |
|                       | -50.00                    | 41             | 16               | 23                     | 2          | 2-72              | 14     | 2 - 89                | 8      |
| <i>S. anatum</i>      | - 0.16                    | 41             | —                | 20                     | 21         | —                 | —      | 2 - 15                | 9      |
|                       | - 5.50                    | 48             | 11               | 30                     | 7          | 2-37              | 18     | 2 - 28                | 10     |
|                       | -67.00                    | 24             | 5                | 19                     | —          | 4-14              | 6      | 2 - 107               | 7      |
| <i>S. newport</i>     | -0.15                     | 6              | 1                | 2                      | 3          | 51                | —      | 9 - 14                | —      |
|                       | -0.38                     | 8              | 1                | 5                      | 2          | 2                 | —      | 4 - 28                | —      |
|                       | -1.30                     | 6              | 3                | 3                      | —          | 9-16              | —      | 7 - 16                | —      |
| <i>S. derby</i>       | -0.13                     | 6              | —                | 3                      | 3          | —                 | —      | 1 - 36                | —      |
|                       | -5.00                     | 12             | —                | 8                      | 4          | —                 | —      | 2 - 11                | —      |
|                       | -15.00                    | 12             | 3                | 4                      | 5          | 4-11              | —      | 2 - 44                | —      |
| <i>S. bareilly</i>    | - 0.12                    | 6              | 1                | 4                      | 1          | 6                 | —      | 1 - 3                 | —      |
|                       | - 1.70                    | 12             | 6                | 5                      | 1          | 2-11              | —      | 2 - 9                 | —      |
| <i>S. pullorum</i>    | 1,300                     | 12             | 5                | 1                      | 6          | 0- 1              | —      | 1                     | —      |
|                       | 7,000                     | 11             | 10               | 1                      | —          | 1- 2              | —      | —                     | —      |
|                       | 16,000                    | 12             | 12               | —                      | —          | 0- 2              | —      | —                     | —      |

<sup>a</sup>Summarized from McCullough and Eisele (24,25,26).

TABLE 7. Evidence for low infectious dosage.

| Year | Product              | Infecting dose |
|------|----------------------|----------------|
| 1967 | Carmine dye (22)     | 15,000         |
| 1970 | Ice cream (9)        | 1,000          |
| 1974 | Chocolate candy (15) | 100            |
| 1975 | Pancreatin (23) (28) | 200            |
| 1975 | Raw hamburger (16)   | 60-2300/100 g  |
| 1977 | <i>S. agona</i> (5)  | Low            |

This is not to ignore industry's direct responsibility for certain outbreaks, viz. *S. eastbourne* in candy (15), the roast beef outbreaks (7), the isotonic diet cases (6) and diseases traced to pancreatin powder (28). In each of these, proper quality control would have detected the problem before the consumer was at risk. Thus industry control is essential if the curve of incidence is to remain at best in a steady or slightly inclined state. If we are serious about the problem, this is not an encouraging prospect. Is there an answer?

### EDUCATION

The NRC Report addressed itself to the need for education, with the admission that practically speaking salmonellae can not be excluded from most raw foods, especially those of animal origin. It urged that all people who prepare foods at home or commercially be educated to render these products safe before serving them to the consumer. The report recommended a continuing massive effort directed toward the foodservice industry. It bewailed the fact that most home economics majors who become high school teachers are poorly prepared to instruct their students in the sanitary practices required to prepare foods safely. It pointed out that with the control of more serious diseases, schools have come to equate personal hygiene with making oneself attractive to others with scant attention to sanitation that must be practiced at home, on the farm, in the factory and

especially in foodservice establishments. It recommended an educational effort to change this passive attitude.

There have been innumerable conferences, symposia, panels and workshops dealing with the problem of human salmonellosis, specifically, and foodborne disease generally. Virtually without exception conclusions similar to those in the NRC Report have been reached.

Government at various levels, educational institutions and industry have taken sporadic and abortive stabs at this problem. These have taken the form of discussions with consumer groups, television and radio broadcasts, newspaper articles and pamphlets. Results have not been successful. As an example, in April 1973 the General Accounting Office commissioned the Gallup organization to determine the number of women who: (a) know what *Salmonella* is, (b) know how to minimize the spread of *Salmonella* that may be present in raw meat and poultry that are brought home, (c) believe that raw meat and poultry are inspected for *Salmonella*.

Results based on 816 women were as follows: (a) 74% did not know what *Salmonella* is, (b) 66% did not know how to minimize the spread of salmonellae and (c) 39% thought that raw meat and poultry are inspected for the presence of *Salmonella* by federal or state inspectors. The Gallup organization claimed that these percentages are accurate within  $\pm 4\%$  at the 95% level of confidence (2).

The increasing incidence of salmonellosis indicates, even more dramatically than the Gallup poll, that our educational efforts have been unsuccessful. Since the epidemiology of the disease has not changed and since we admit that it is not possible to eliminate salmonellae from raw foods, the future looks as bleak as the past.

One potentially effective route to consumer education has been largely ignored. What would the long term effect have been if in 1969 a massive program of education had been directed toward students in secondary schools, a program at the lower levels directed toward the elements of personal hygiene, this leading to education in proper food preparation and handling practices. Such an educational program would in fact teach grammar and high school students what food poisoning is all about, what causes it and how it can be

prevented. This is not to suggest a one-time course taken at one stage in the child's development, such as driver education. It is to suggest a course of study that would be started early in the child's education and would continue through the high school years with increasing sophistication. If we had done such a thing 10 years ago we would now have half a generation within the population that is knowledgeable in proper food handling practices and this certainly would have an impact on the incidence of *Salmonella* and other foodborne illness. If something of this sort is not done, then the individual chosen to discuss "*Salmonella* 20 Years Later," will be forced to extend the same curves and enlarge the tables currently used. Obviously the problem that exists today existed 10 years ago. Such courses of study have not been developed; we do not have teachers with training to handle them. But this could be done with proper governmental support and impetus. Were we to have half a generation trained in proper food handling practices, its influence on its elders might have a greater impact than the current pamphlets and TV spots.

There is yet another area in which something could be done, and if it is not done, the future will be as bleak as the past. This has to do with the control of food handling in foodservice establishments, including hotels, restaurants, food markets and institutions. Included among these are caterers. Here there is no dearth of educational materials, for well-written codes have been prepared and are readily available. The problem is that they are not followed, not understood, not enforced. Where might we be today if 50% of the USDA and state meat inspectors, engaged in continuous on-the-line inspection in meat plants, had been diverted to inspection of foodservice establishments? Obviously, continuous inspection of the thousands of such establishments is unfeasible. But, regular inspections by knowledgeable personnel with authority to enforce codes would effect improvement if punitive measures were the alternative to non-compliance. Why not divert manpower engaged in continuous inspection of meat processing establishments to the area where the need exists? For other reasons, the Booze-Allen report (3) on the USDA meat and poultry inspection recommended less on-the-line inspection. If

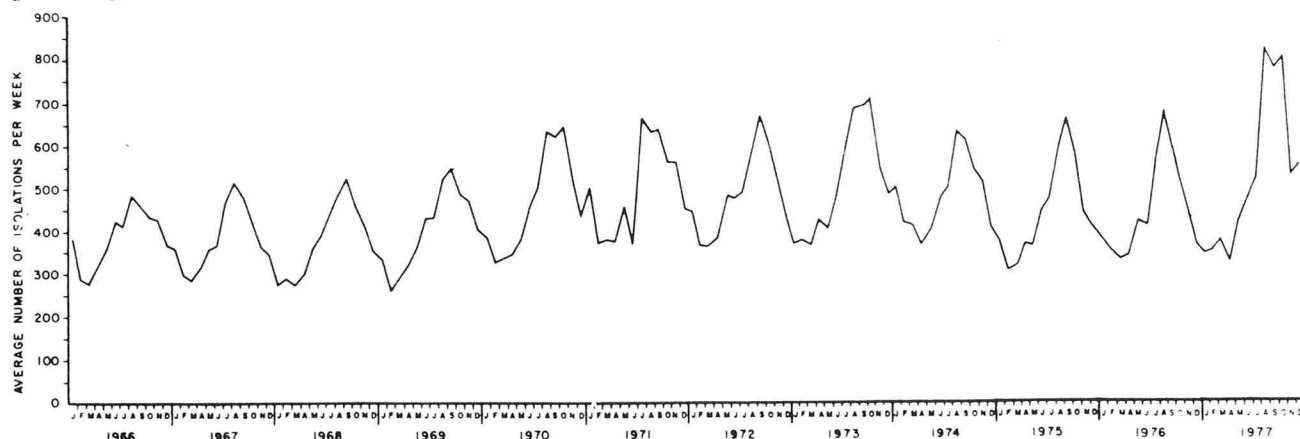


Figure 3. *Salmonella* surveillance program; reported isolations from humans by months, United States, 1966-1977 (4).

that manpower, 10 years ago, had been diverted to foodservice establishments, there is no doubt that the incidence of foodborne illness would have been significantly less than we see today. The bulk of regulatory activity is being exercised over food processing rather than in the areas where foods are used. It is in the preparation areas where the bulk of the problems develop, and professional food handlers should be subject to regulation at the expense of less on-the-line inspection of processors.

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## Effect of Light on Alteration of Nutritional Value and Flavor of Milk: A Review<sup>1</sup>

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### ABSTRACT

Milk in glass, polycarbonate, high density polyethylene, blow-molded polyethylene, plastic bags and paperboard containers, when exposed to fluorescent light or sunlight, will develop a characteristic off-flavor described synonymously by many researchers as activated, sunlight or oxidized flavor. The extent of flavor development is related to the exposure interval, strength of the light and amount of milk surface exposed. Paperboard containers, particularly those with large printed areas of dark ink or foil in the laminate, offer the best protection to milk, while the remaining containers offered limited protection at best. Characteristic flavor is produced principally by conversion of methionine to methional. Partial loss of vitamins B<sub>2</sub> and C and some amino acids parallel development of light-induced off-flavor. Loss of other constituents is minimal. Many researchers offered suggestions to improve the flavor stability of milk held in display cases illuminated with fluorescent lights. Among these are changes to more protective packaging, reduction of radiant energy to 538 lux, use of gold or "bug light" types of fluorescent lights and more dark ink areas on exposed parts of paperboard cartons (gables and main display panels). Perhaps grocers should bag all containers of milk to afford adequate protection from sunlight between points of refrigeration.

The change of flavor of milk by light was first reported in Europe in 1890 and subsequently by others (44). Since that initial report, both trade and technical journals have published considerable supportive information on this topic. This review focuses attention on the effects of light on both the nutritional value and flavor of milk. Also consideration is given to the packaging system used to hold milk between processing and consumption.

For purposes of this review, many synonyms are used to describe light-induced off-flavor. Some of these are "activated flavor," "oxidized," "sunlight flavor," and old literature refers to "burnt feathers" as a typical flavor. Regardless of the term, these are used synonymously in this paper. The problem of light exposure has changed with modern times. Formerly, milk was left on doorsteps; currently, with the decrease in retail route delivery, most milk is purchased from stores. It is in this location that exposure to fluorescent light becomes a significant problem. Moreover, in conveying milk products from store to home in transparent or translucent containers, a further flavor problem could manifest itself.

### ALTERATION OF FLAVOR

Acceptance of milk as a food is based to a large extent on its flavor and shelf-life. Barnard (4) reported that the incidence of oxidized flavor in fluid milk was increasing rapidly. During a 4-year-period, over 1,600 milk samples collected from 400 retail stores in Pennsylvania and representing 250 brands were analyzed by a trained taste panel. Initially, in 1967, only 6.7% of 210 samples analyzed were criticized for oxidized flavor. This percentage increased to 23.9% or 106 of 443 samples in 1970. To further illustrate the breadth of the oxidized flavor problem, a comparison was made of four major types of containers and the distribution of oxidized flavor: blow-molded plastic containers showed that 86.1% of milk contained was criticized as having an oxidized flavor, plastic bags showed 50.0%, glass bottles showed 46.4%, and the least was plastic coated paper with 12.7% having oxidized milk (4). It is apparent from this literature that development of light-induced off-flavor is clearly a function of the amount of radiant energy at the milk surface and the length of exposure.

#### *Amount and source of light necessary to produce flavor alteration*

When the effect of light on milk flavor was first observed, sunlight was the only energy source that induced this oxidized or activated flavor. Needless to say, accurate measurement of sunlight intensity was difficult since it varied with season, geographic location, cloud cover and time of day (44). Holmes and Jones (26) used a pyrheliometer to record and control its intensity. Other more recent researchers have attempted to correlate the intensity of fluorescent light with the development of objectionable oxidized flavor. Dunkley et al. (16) showed that oxidized flavor development was a function of exposure time and distance from the light source. Using two 40-watt Cool White (F40CW) fluorescent lamps, 1.22 m long, and 1 quart clear flint glass bottles, they proved that exposure time for comparable light activated flavor in milk was 5 to 6 times longer at a distance of 30.5 cm from the light source than at 1.3 cm. Data showed similar and perceptible activated flavor developed in 20 to 40 min at 1.3 cm from the light and 2 to 4 h at a distance of 30.5 cm.

Bradfield and Duthie (8) reported variations in light intensities from 215-5380 lux in display cases in

<sup>1</sup>Contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

Vermont, while de Man (13) stated that fluorescent light intensities in supermarket display cases in Guelph and Toronto, Canada varied from 550-5500 lux with many in the range of 1000 to 3000 lux. In another study, the average light intensity in display cases in 105 supermarkets was 2001 lux (2). Hedrick and Glass (22) attempted to simulate average conditions in a grocery store display case, using fluorescent light adjusted in energy output to 1614 lux and positioned with 5.1 cm from the top of milk containers. Intensity of this magnitude caused changes in flavor and composition after milk was exposed for 5 h in both paperboard and blow-molded plastic gallon containers. In a similar test, Dimick (14) used a retail milk display case illuminated by cool white fluorescent lamps (F40CW) mounted parallel to the shelves and 45.7 cm from the containers. Illumination as measured with a Weston illumination meter averaged 1076 lux at a position perpendicular to the light source and at the midpoint of the exposed vertical surface of the container. He used paperboard (unprinted, plastic-coated paper and 0.58 mm thickness), blow-molded polyethylene (55 g and 0.52 mm thickness) and clear flint glass (2.5 mm thickness) half-gallon containers with each type of container having 185 to 190 cm<sup>2</sup> of surface area exposed to the light. Average light transmission in these experiments was 2.8% for paperboard, 69.2% for plastic and 90.7% for glass containers. Subsequently, flavor comparisons were made using a trained panel of 12 women. After 12 h of exposure all containers of milk were rated lower than was unexposed control milk. Flavor of milk stored in plastic and glass containers was similar and decreased markedly in acceptance after 12 h of exposure, while milk in paperboard containers required exposure to the light source for 48 h to reach similar flavor ratings.

In a second experiment, Hoskin and Dimick (27) evaluated the ability of returnable polycarbonate, tinted polycarbonate, high density polyethylene, flint glass containers and nonreturnable unprinted paperboard cartons to protect homogenized milk from activated flavor development. After 12 h of exposure to cool white fluorescent lamps (F40CW) producing 1076 ± 50 lux at the vertical container surface, significant differences in milk flavor scores were apparent. Milk held in paperboard containers had hedonic flavor ratings showing no difference statistically to non-illuminated control milk. Milk in glass, high density polyethylene and non-tinted polycarbonate containers was afforded little protection, while milk held in polycarbonate containers tinted with a blocking material effective against energy in the 380-480 nm region had hedonic flavor ratings intermediate to those given for milk in paperboard and for milk in glass, etc.

As little exposure time as 10 min will produce a discernible activated flavor (26). However, intensity of off-flavor diminished with prolonged exposure (26). Presumably, this is attributed to decomposition of methional developed during subsequent and prolonged

exposure to light.

One solution (3) was to minimize the amount of lighting in retail cabinets to an intensity of 538 lux. An alternative was to use gold or "bug light" fluorescent lights in display cabinets (16).

#### *Chemical changes occurring to exhibits this flavor*

Many attempts have been made to identify the characteristic flavor compounds in milk having a sunlight or activated flavor. Weinstein and Trout concluded that a protein in heat-treated whey would produce the characteristic flavor (48). Shortly after this report, Patton and Josephson (35) showed that the amino acid, methionine, was responsible for activated flavor in milk. Using a pure solution of methionine, they detected a flavor after sunlight-exposure similar to that observed in skim milk also exposed to sunlight.

Samuelsson and Harper (36) demonstrated the role of Strecker degradation in converting methionine into methional, ammonia and carbon dioxide. Vitamin B<sub>2</sub> and oxygen are needed in this reaction. Moreover, they suggested that free amino acids were also necessary for this reaction. McLaren (32) found that 45 kcal of energy/mol were required to split the peptide linkage to free amino acids. In the visible region of the light spectrum, energy available is 71 to 95.3 kcal/mol, which is more than sufficient. Further, McLaren showed that 58 kcal/mol were necessary to split a C-S bond to form a mercaptan. Using this evidence (32), Samuelsson and Harper (36) proceeded to demonstrate the possible formation of methional, hydrogen sulfide, formaldehyde, acetaldehyde, propional and various combinations of hydrocarbon substituents on sulfhydryl, sulfide and disulfide bases. However, Cohen and Ojanpera (11) indicated that the photoreduction of methionine at pH 7 produced methional with a 100% yield.

Additional evidence showed that methionine was oxidized to methional in presence of direct sunlight (1) and the proposed mechanism by which this reaction proceeded (11). Prolonged exposure to sunlight produced such obnoxious flavor compounds as methyl mercaptan. It is apparent from this research that factors responsible for the degree of off-flavor are three-fold: intensity of the light source, duration of exposure and compounds generated (1).

Singleton et al. (41) suspected that a component of relatively large molecular weight rather than methional was responsible for the flavor. Speculation occurred because of a nondialyzable component found in skim milk following addition of riboflavin and exposure to sunlight. In a model system containing tryptophan and riboflavin, a complex was formed upon exposure to light which was the suspected flavor component. Moreover, Bassette (5) confirmed the effect of light on production of some volatile compounds in milk exposed to sunlight. He found increases in compounds which normally comprise the flavor in milk, such as acetaldehyde, propanal, methyl sulfide, acetone, butanone, n-pentanal and n-hexanal. Apparently the precursor to acetaldehyde is

in the nonfat portion while precursors to other carbonyl compounds are found in the lipid phase.

#### *Threshold of flavor perception*

Patton stated that the threshold for perception of methional, the principal entity in sunlight flavor, was 50 parts per billion (34). In skim milk there is 1.3 to 4.3  $\mu\text{m}$  of free methionine per liter which represents a potential for methional of 3 to 9 times the taste threshold.

#### *Effect of processing variables on development of flavor*

Raw milk is more susceptible to development of sunlight flavor than milk that has been thermally processed (43). Weinstein and Trout (47) showed that heating milk to 80 C and holding for 5 min did not affect development of sunlight flavor. However, they did observe that sunlight flavor tended to destroy the heated flavor.

One would anticipate that the freeing of sulfhydryl groups in whey proteins by excessive heat treatment would reduce the rate of development of this off-flavor since these sulfhydryls are highly sensitive to and readily combine with oxygen. In fact, this practice of high heat treatment has been used for decades to retard onset of oxidized flavor in susceptible and high-fat dairy foods.

Before using vitamin concentrates to fortify milk with vitamin D, irradiation with ultra-violet light was practiced. "Irradiation flavor" was found to be the result of using early equipment (10). Weckel and Jackson (45) first reported this flavor as a part of their historically significant efforts to produce Vitamin D-fortified milk by irradiation using carbon arc lamps. Later, Flake et al. (18) isolated a sulfur-containing material thought to be the material responsible for the off-flavor.

Homogenization increases the susceptibility of milk to oxidized flavor and this has been reported by many (17,24,47). Also, in excess of 95% of fluid milk sold in the United States currently is homogenized.

#### *Influence of the wavelength of light*

Sunlight flavor was an important defect in milk irradiated to increase its vitamin D content. Flake et al. (17) studied the effect of different wavelengths of light and showed that elimination of wavelengths below 460 nm reduced the rate at which activated flavor developed. Later, Herreid et al. (25) found that ruby glass gave almost complete protection against sunlight flavor development since no radiant energy below 600 nm was transmitted through the glass. In addition, the rate of development of activated flavor is reduced in those retail cabinets equipped with yellow fluorescent lights or fluorescent lights with yellow shields. Radiation spectra of these lighting systems show minimal energy emitted below a wavelength of 540 nm.

#### *Influence of milk temperature*

In two different experiments, Dunkley et al. (16) proved that higher milk temperatures increase the intensity of the light flavor. Using temperatures of 0 and 11 C and later 1 and 16 C, they identified that the flavor

problem was attributed to both a faster reaction rate and a more intense exposure.

#### *Effect of storage time on flavor intensity*

One of the greatest contributions to development of activated flavor is the length of storage in illuminated cabinets or in sunlight. In some vertical dairy cases, there are locations from which milk containers are seldom removed, for example, toward the back and center part. Milk containers in these areas are sold, in general, only when moved elsewhere in the cabinet (8). Also, in the low, reach-in variety of retail cabinet, the front row of cartons may not be sold for long periods, while containers in rows 2 and 3 were generally removed at a much faster rate. For example, in three stores examined by Bradfield and Duthie (8), half-gallon milk containers in row 1 disappeared in the amount of 27%, while similarly sized containers in row 2 and 3 disappeared in amounts of 32 and 22%, respectively. On the other hand, quarts disappeared from row 1 in the amount of 16%; row 2, 25%; and row 3, 20%. Rows of both quart and half-gallon containers further back in these cabinets were sold more slowly.

In an in-depth study published by Market Facts of New York (2), 105 retail milk outlets were evaluated for disappearance of milk from cabinets as a means of assessing the length of exposure to fluorescent lighting in these display cabinets. The average light intensity in these stores was 2001 lux. In 15 outlets in each of 6 cities, using time-marked milk containers, they found 71% of these containers unsold after 5 h, 58% unsold after 8 h and 37% unsold after 24 h regardless of container size or type. The study involved use of 58,973 marked containers.

Dunkley et al. (16) found that 2 days of storage in the dark followed by exposure to a known amount of radiant energy resulted in less activated flavor than did a similar exposure immediately following processing. These researchers had no explanations for this effect.

### **EFFECT OF MILK CONTAINER ON OFF-FLAVOR DEVELOPMENT**

The type of container used to hold milk between processing and consumption is extremely critical in minimizing flavor and nutrient alteration. Considered in this section will be glass, returnable high density polyethylene, returnable polycarbonate, blow-molded polyethylene, paper board and plastic bag containers.

#### *Glass*

In 1920, Hammer and Cordes (17) suggested that brown-colored glass milk bottles were effective in preventing the action of light on milk. Later, Herreid et al. (22) showed that amber glass offered protection from light for intervals up to 30 min, whereas ruby glass containers offered the best protection of all containers, including paper. Ruby glass permitted minimal light transmission in wavelengths less than 600 nm. These researchers found that milk taken from pasture-grazed

cows showed no activated flavor in ruby glass containers after 2 h in direct sunlight. On the other hand, milk in amber bottles showed only slight off-flavor after 30 min in sunlight. In all trials to determine the protective effect offered by various colors of glass, comparisons made to clear glass indicated that it offered virtually no protection from the actinic rays of the sun or fluorescent light. The lack of or negligible protective effect offered by clear glass has been reported by numerous authors (14,16,22,25,37,42 and 49). In fact, Henderson et al. (24) found sunlight flavor in milk exposed 10 min in the sun at noon. In the 380 to 480 nm critical region, glass containers (3.4 mm wall thickness) showed 95% energy transmission (27).

Because of the problem of sunlight flavor in milk, many retail home delivery companies formerly supplied consumers with insulated metal boxes to preclude action of the sun's radiant energy. In the 1930's, little off-flavor was observed in creamline type of milk. Later, homogenization became widely practiced and the problem surfaced to a substantial degree. Colored or tinted bottles were used by some dairies in an attempt to alleviate the flavor problem. However, very few dairies, if any, used ruby glass because of cost; most used amber bottles. The amount of protection offered even by amber glass was insufficient and their use waned.

#### *Blow-molded single service polyethylene container*

Single-service, plastic containers allow some additional protection from sunlight because of the opacity of the plastic. Dimick (14), using blow-molded half-gallon containers 55 g and with 0.52 mm wall thickness, found that homogenized milk developed off-flavor attributed to light at a rate close to that of milk in glass containers. An expert taste panel ranked milk exposed to fluorescent light in glass and blow-molded plastic containers for 12 and 24 h as "dislike slightly" with a hedonic rating of 4.0. The panels used a 9-point hedonic rating system.

Barnard (4) indicated that single-service plastic containers provide little more protection for milk to the actinic effect of light than glass. In a survey of market milk conducted in 1970, 86.1% or 31 of 36 samples in blow-molded plastic were oxidized. A similar result, 84.2%, was indicated in sampling in 1973. Blow-molded plastic containers permit 25 to 50% of the light to pass through the wall. Plastic 3-quart milk containers allow 60% light transmission at 400 nm and over 80% at 700 nm. Some solutions were offered to reduce transmission; add such materials as titanium dioxide, talc, and other agents to block visible light. However, de Man (13) recently showed the negligible effect of using titanium dioxide in bottle matrix.

Hansen et al. (21) established that homogenized milk in blow-molded plastic containers in a simulated display case showed sunlight flavor after 2 to 4 h of exposure to 2152 lux of energy produced by 40-watt Cool White fluorescent lamps 6.4 cm from the containers. Barnard et al. (3) found an increased time, 5 to 8 h, to develop sunlight flavor when the lamp intensity was 1937 lux,

while Dimick (14), using 1076 lux of fluorescent light, found homogenized milk in blow-molded containers with sunlight flavor after 12 h of exposure.

#### *Paperboard containers*

Of all materials used for single service milk containers, paperboard best protects milk's flavor and nutritional qualities. It is not without fault, as reported. However, some researchers offer suggestions to yet improve the light absorption and reflection.

Henderson et al. (24) first studied the effects of sunlight on milk in different types of paperboard containers. They used (a) white bleached paper, the thinnest of the three types; (b) cream colored paper of intermediate thickness; and (c) multiple layers of bleached white outer plies and unbleached and light brown inner plies, the thickest of the three paperboards considered. These paperboards were parafined, which should be similar to the current paperboard which is polyethylene-coated. A Weston photometer placed behind the paperboard exposed to direct sunlight gave readings of a-400, b-180 and c-80. All paperboards used in this study were much superior to glass. Milk stored in cartons made from material (a) yielded a slight sunlight flavor in 1 h in noon sun, milk in carton (c) showed none, while milk in carton (b) was scored intermediate for off-flavor but showed no sunlight flavor.

Unprinted portions of commercial milk cartons differed markedly in amount of light transmission (11,13). Ink used absorbs light energy, dependent upon the color of the ink. The conclusion reached from this effort was that cartons should be designed with large areas of red, brown, black, yellow or orange inks to absorb the shorter wavelengths of energy. This is particularly true of the top or gable of the carton. Bradfield and Duthie (6,7) reported values for light energy transmitted through paperboard colored with red, blue, black and green inks. With an uncolored paperboard carton established as 100% transmission reference, using a 4304 lux fluorescent light then red gave 54% transmission, blue 27%, black 27% and green 18%. Bradfield and Duthie (6,7) stated that milk in uncolored cartons exposed to 2376-2690 lux of fluorescent light changed flavor in 54 h. At 4304 lux, milk in the uncolored cartons developed sunlight flavor in 12 to 18 h. When a green carton was used, the time for onset of the off-flavor was 18-24 h (7). Sattar and de Man (37) showed that light transmission values for paperboard containers at 400 nm was 0% and about 13% at 700 nm. Printing on the carton reduced energy transmission values to 0 to 10%.

It is apparent that any paperboard laminate that contains an aluminum foil layer, such as used for long life dairy products, would minimize the amount of light transmitted and increase the flavor shelf-life of the product contained.

#### *Plastic bags*

It is questionable whether this type of container should

be considered principally for such reasons as: number for individual consumer use, uncertainty of the presence of an outer protective cardboard box, i.e., bag-in-box container and the mechanism or manner of storage. Barnard (3) gave the only data with no mention of any outer wrap. In this study, 50% of samples in plastic bags (6 of 12) were oxidized. This value is comparable to that for oxidized off-flavor in milk in glass, 46.4% or 26 of 56 samples.

#### *Returnable high density polyethylene and polycarbonate containers*

These milk containers are relatively new and have shown limited consumer appeal. Hoskin and Dimick (27) showed that gallon-sized, high density polyethylene milk containers with a 1.7-mm wall thickness allowed transmission of 58% of fluorescent light, as measured using a Weston model 756 meter. Gallon-sized polycarbonate milk containers with a 1.5-mm wall thickness allowed 90% transmission while those polycarbonate containers structured with energy-blocking material allowed 75% transmission through a 1.5-mm wall.

#### **EFFECT OF CONTAINER TYPE ON CHANGE IN COMPOSITION OF OTHER MILK CONSTITUENTS**

In consideration of the direct effect of light on flavor of milk, it is apparent that effects on other milk constituents would be proportional and related to wavelength, intensity and duration of exposure to light.

#### *Riboflavin*

In sunlight and fluorescent light, loss of riboflavin in milk is rapid. Herreid et al. (25) indicated that in 30 min as much as 30% of riboflavin in milk was destroyed with this going to 80% in 2 h of exposure to sunlight. Singleton et al. (41), in a similar study, showed 64% loss of riboflavin in 30 min of exposure to sunlight and 89% loss after 2 h. Degradation followed first order kinetics and the rate increased directly with temperature (39). Amber bottles gave complete protection and prevented destruction of riboflavin.

Since the rate of destruction of riboflavin is related directly to milk temperature as well as the amount of light transmitted through the container, greater loss would occur in summer months when product temperature generally would be higher (42). Dunkley et al. (16) showed that destruction of riboflavin in milk retail cabinets was directly related to the wavelength and wattage or food candles of energy. Riboflavin is most labile when exposed to radiant energy between 415 and 455 nm (39). At wavelengths above 550 nm, destruction of riboflavin was markedly reduced (16). Further, light-activated flavor was induced by light of more wavelengths than that absorbed by riboflavin. Josephson (28) showed that light above 550 nm contributed to off-flavor, while Dunkley et al. (16) proved that wavelengths less than 550 nm were responsible for loss of riboflavin. Sattar and de Man (37) indicated that the

most damaging wavelengths are 350-500 nm. The use of a gold lamp or "bug light" color would be one solution to control of both off-flavor and vitamin destruction in milk stored in retail cabinets (16). Maniere and Dimick (31) showed that the rate of riboflavin destruction increased when it was in a free form and unassociated with the proteins or fat in milk. A recent study (33) chemically defined the major degradation product of riboflavin as lumichrome.

#### *Ascorbic acid*

Milk nutritionally is not noted as a significant source of vitamin C. Ascorbic acid is rapidly oxidized to dehydroascorbic acid and this reaction is accelerated in the presence of light (44). The rate of destruction of vitamin C is proportional to the amount of light transmitted through the container, the wavelength of that energy (25) and the presence of riboflavin (40).

Henderson et al. (24) indicated that brown or amber glass and paperboard containers offer the greatest protection from the actinic rays of radiant energy. Using paperboard containers with different light opacities, they showed that vitamin C destruction was proportional to light transmission and was indirectly related to flavor development. Hedrick and Glass (22) showed similar results using paperboard and blow-molded plastic milk containers.

Woessner et al. (49) showed that ascorbic acid was stable during normal thermal processing operations but disappeared rapidly during light exposure. Dunkley and others (16) analyzed the active wavelengths of light that would destroy ascorbic acid. They showed that energy between 400 and 550 nm was responsible. As with riboflavin, the greatest protective effect in illuminated retail cabinets would be from gold or "bug light" type lamps.

#### *Amino acids and proteins*

Dimick (14,15) showed that even though the sunlight flavor was attributed to oxidation of the essential amino acid, methionine, no significant change occurred in its concentration or the concentration of 16 other amino acids over a period of 144 h when milk in paperboard, blow-molded plastic and glass containers was exposed to fluorescent light. Singleton et al. (41) and Gregory et al. (19) found that the amount of tryptophan decreased on exposure of milk to light and this loss was directly related to loss of riboflavin and indirectly related to sunlight flavor intensity. After 2 h of exposure to sunlight about 15% of the tryptophan disappeared. Cysteine, histidine, methionine, tryptophan and tyrosine are principally susceptible to photosensitized oxidation (2).

It is possible that photodegradation of isolated milk protein fractions may occur (29,30,46). Both low and high molecular weight serum proteins are involved as substrates.

#### *Vitamin A and $\beta$ -carotene*

Sattar et al. (38) showed that loss of vitamin A and its precursor could be markedly reduced by limiting



exposure of milk to energy below 465 nm. Destruction was not autocatalytic and followed zero-order kinetics. No synergism was observed except that at  $\beta$ -carotene concentrations of greater than 2.5  $\mu\text{g}/\text{ml}$  a protective effect on vitamin A was observed.

### FLAVOR EVALUATION BY TASTE PANELS

Coleman et al. described the problem best (12). In most light flavor research, trained or expert panels use the official A.D.S.A. score card for milk, making it difficult to compare consumer acceptability of light-activated flavor. In their study, experts initially flavor-scored milk, then a panel of trained tasters was used, followed by mass sampling by untrained people to assess their ability to discriminate light-activated flavor. The consumer taste panel of 781 people (15-25 years old) rated three samples of milk, one exposed to 1076 lux of fluorescent light at 7 C for 12 h, a second sample exposed similarly for 24 h and the third sample was a non-light exposed control sample. A 5-point hedonic scale was used by 391 panelists ranking acceptance as control-best, then the milks exposed 12 and 24 h, respectively. A similar placing resulted when the remaining 390 panelists used a ranking procedure. Coleman et al. (12) showed that paperboard is the most acceptable container for milk. However, the consumer is only able to differentiate the flavor of milk samples when comparisons are made. Further, consumers might not object to the off-flavor unless comparison samples are available. Bray et al. (9) confirmed these results in a 200 heterogeneous consumer survey. Furthermore, no significant difference was found between age groups (a - younger than 25; b - older than 25) in ability to distinguish oxidized milk from non-exposed control milk; however, in all groups, females showed a better ability to distinguish between the two samples offered than did males.

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## IDF Report, *con't from p. 335*

original statement discussing additional assessments was deleted. In the future, a draft will be prepared as to the cost of membership when the U.S. decided to become a formal member of IDF.

The participants at the meeting agreed to pay the \$100.00 membership fee. The treasurer was authorized to open an account and have the necessary stationery printed. This money will be used to honor vouchers for expenses accrued in operation of the Interim Committee.

The obtaining of operating funds for a permanent committee was reviewed and it was recommended that at this point, no government funds be solicited but an attempt could be made to obtain some financing from various foundations.

Respectfully submitted,

Harold Wainess  
IAMFES Representative  
International Dairy Federation

### Proposed Functioning of an Interim United States National Committee of the International Dairy Federation

1. The name of the organization shall be the "Interim National Committee of IDF in the USA."
2. The Committee shall be the member organization for the United States of America

in the International Dairy Federation until December 31, 1980. IDF membership fee shall be waived for this period.

3. The purposes and objectives of the Committee are:

- a. to assess the need for a permanent United States National Committee of IDF;
- b. to provide a means of representation for those likely to benefit from or contribute to the purpose and activities of the IDF and of the Committee;
- c. to organize effective United States participation in and contribution to the work of the IDF;
- d. to provide effective communication and utilization in the United States of the results of the work and activities of the IDF.

4. The members of the Committee shall be persons involved in the United States dairy industry interested in the objectives and purposes of the Committee and of the IDF.

- a. Membership may be on a personal basis, or as a representative of an organization, institution or association.
- b. Members shall pay an initial fee of \$100.
- c. A prospective member shall, upon written notice and payment of initial fee to the Committee Secretary, become a member for the duration of the Committee's existence.

5. The Committee shall select a Chairman, a Secretary who will be responsible for communication between the IDF and Committee members, a Treasurer, and any other officers necessary to further the objectives and purposes of the Committee.

6. Meetings of the Committee shall be held from time to time at the call of the Chairman

which all members are entitled to attend. Proceedings of all meetings shall be communicated to members.

7. Liaison advisory groups may be named by the Committee. These groups shall consist of members and/or non-members who represent various institutions, organizations, associations, or government and whose views and expertise will further the objectives and purposes of the Committee.

8. The disbursement of funds of the Committee shall be made in furtherance of the objectives and purposes of the Committee. Expenses incurred in participation at any meeting of the Committee, liaison advisory groups or of the IDF by any individual shall be paid by that individual or his organization, unless otherwise indicated by the Committee.

9. United States participation in the work and activities of the IDF or any of its Commissions shall be under the direction of the Committee.

10. Commission representatives shall be expert advisors to the Committee who may represent the Committee at IDF meetings, act as Chairman of ad hoc subcommittees and otherwise assist the Committee.

11. Bylaws may be adopted or amended at any meeting of the Committee provided thirty days notice has been provided and a copy of the proposed amendment has been included with the notice.

12. The Committee shall issue a report no later than April 1, 1981 reviewing its previous work and its assessment of the need for an official, permanent United States National Committee of IDF.

## Status of the Model Retail Food Store Sanitation Ordinance

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### ABSTRACT

This is a status report on the Model Retail Food Store Sanitation Ordinance developed by the U.S. Food and Drug Administration for adoption by State and local regulatory agencies. The Ordinance is currently under final review by the Federal agency. Approval is expected by the end of 1979. It is the first time the Federal Government has developed specific recommendations for the sanitary operation of retail food stores. The Ordinance has been under development since 1973. It contains information on some General Provisions, Food Care, Personnel, Equipment and Utensils, Cleaning, Sanitization and Storage of Equipment and Utensils, Sanitary Facilities and Controls, Construction and Maintenance of Physical Facilities and Compliance Procedures. Several drafts preceded the original proposal which was available for public comment in October, 1977, with a subsequent revised edition available for comment in November, 1978. All comments received were given due consideration and the final version prepared for agency clearance. FDA believes the Ordinance is practical and enforceable. If the recommendations are implemented by the retail food store industry and equitably enforced by the regulatory agencies, the marketplace should be a safer place in which to retail food which meets the expectations of the buyer.

This paper is an update on "The Status of the Model Retail Food Store Sanitation Ordinance" developed by the U.S. Food and Drug Administration and recommended for adoption by state and local regulatory agencies. To appreciate where we've been in the development of the document, I'd like to share with you its history.

Interest in an ordinance for the retail food store industry was initiated as the result of the 1971 Conference on Food Protection in Denver (4). Regulatory agencies and the industry expressed their concern to FDA following that conference about developing such a model ordinance for the retail food store industry, estimated to be approximately 265,000 establishments.

There have been model ordinances developed in the past by FDA and only recently revisions were made to the sanitation manuals for the food service (1976) and vending industries (1978). There has never been any federal sanitation criteria written specifically for the retail food store industry. However, some State and local enforcement agencies have developed their own guidelines for checking the sanitation level of the stores in their respective jurisdictions. Traditionally, the States and local regulatory agencies inspect retail food stores, rather than FDA.

Because of our past experience in the development of ordinances, it seemed logical that we should undertake the task of providing a uniform model ordinance that was consistent in recommendations with previously developed documents for the foodservice and vending industries.

The publication of this particular model ordinance will complete a triad of documents listing recommendations of FDA for the sanitary operation of retail food establishments (foodservice, vending, and retail food stores).

### DEVELOPMENT OF THE ORDINANCE

As a result of the Denver Conference, followed by industry representative contacts and requests from the regulatory agencies, the Division of Retail Food Protection initiated a literature search to obtain additional information and to confirm the need for a model ordinance for the retail food store industry.

We learned that the Center for Disease Control in the mid 70's had reported cases of trichinosis from adulteration of commercial beef with pork purchased at the retail level (2). The American Medical Association reported in 1972 that a significant proportion of reported foodborne illnesses were attributed to market-prepared, ready-to-eat foods (3). A retail food survey in 1972 revealed the presence of pathogenic organisms in salads and sandwiches (5). Fecal contamination of fruits and vegetables was reported in one literature review (6).

In 1973 FDA looked at 35 State legal documents that contained sanitation requirements used by those States when inspecting retail food stores. Few States were found to have specific sanitary requirements for operation of these stores. Some States used the same specific sanitation criteria when inspecting food service establishments and retail markets. Others used their State Food, Drug and Cosmetic Acts or Regulations based upon those laws. Differences in food temperature requirements, as well as other inconsistencies, were noted between the food service and retail food store codes within the same jurisdiction.

State and local regulatory personnel had reported the most common occurring violations in the marketplace to be the presence or evidence of rodents and insects, lack of adequate or proper equipment for the maintenance of safe food product temperatures, poor hygienic practices

and improper cleaning and sanitizing of food-handling equipment and utensils. The turnover of store personnel and the use of temporary or part-time workers was another important factor that contributed to many observed violations. This pointed out the need for training of all food handlers.

With this background of reported foodborne illness, food sample surveys revealing product adulteration, a variety of State sanitary requirements, little uniformity in enforcement practices and the concern of the industry and regulatory agencies, FDA was convinced there was a need to develop a model ordinance for the sanitary operation of retail food stores.

The ordinance proposal was discussed with representatives of the Association of Food and Drug Officials (AFDO) and industry representatives from the Food Marketing Institute, the National Association of Retail Grocers of the U.S. and the National Association of Food Chains, among others. In August, 1974 AFDO submitted to FDA its own version of a model ordinance and asked that it be published in the *Federal Register* for adoption by the States. The AFDO proposal was quite similar to the FDA draft being developed at the time. FDA had used suggestions from the industry and regulatory agencies, including the USDA, in putting together its proposed ordinance. Some field testing of the inspection report form was done during this period.

In 1973 and 1974, there was considerable discussion about FDA issuing both a regulation and a model ordinance. In fact, we were writing both at one point. A decision was made near the end of 1974 that since FDA would not inspect or regulate retail food stores, there was no need for a regulation. These discussions resulted only in delay of the ordinance, but we believe it was worthwhile to solve this problem of dual publication.

At the time we were developing the ordinance, industry was doing its thing. The trade associations had shown great interest in the ordinance and contributed much to its content. Two trade associations developed training programs incorporating some basic public health principles. The National Association of Retail Grocers (NARGUS), for example, developed and published a retail food store sanitation program with the hope of improving the total environment among the various segments of the food supply chain (1). NARGUS wanted to illustrate that an efficient marketing system can be accomplished and was interested in effecting improvement of food handling, storage, and distribution.

The Food Marketing Institute (FMI) developed a training program for management at the retail level called MUST, the acronym for Manager Uniform Sanitation Training. This is a program to Train the Trainer in instructing those persons responsible for making others aware of their obligations with regard to good food-handling practices in the marketplace.

Early in 1976, a draft of the proposed ordinance was sent to industry representatives and State regulatory agencies for their review and comment. The suggestions

received were incorporated into a final draft with an announcement of its availability for public comment published in the *Federal Register* in October, 1977.

#### COMMENTS ON THE DOCUMENT

We received some very constructive comments from the industry, trade associations, regulatory agencies, individuals, academia, professional associations and the military. Some respondents expressed concern about protection of food while in transit, manual cleaning and sanitizing procedures and our omission of any discussion of sample demonstrations or sales promotions that occur at the retail level. Industry was particularly concerned that the ordinance would be "liberally construed and applied", which in their opinion was favorable to the regulatory agency and detrimental to the industry. Additional comments were made about the compliance portions of the ordinance. Because of the large number of comments received (approximately 1,000), the Commissioner of Food and Drugs concurred that a revision of the proposal would be in order. A revised proposal was prepared, taking into consideration the comments that had been received.

In November, 1978, a second *Federal Register* announcement was made asking for public comment on the revised model ordinance. This comment period ended January 31, 1979.

We received 672 comments from 49 respondents about the revision. Subject areas most commented upon were concerned with some of the definitions (modifications, additions, deletions), food storage, particularly refrigerated storage, manual cleaning and sanitizing operations and some of the compliance procedures dealing with suspension of permits, correction of violations and penalties. There were even some complimentary comments on the proposal which were good to receive.

Remember, the document is an ordinance, not a regulation. It only is effective when adopted by the regulatory agency having responsibility to inspect the retail food store industry in its respective jurisdiction.

We realize that most States and local regulatory agencies have their own legal guidelines that must be followed. However, there are some agencies that want guidance in the compliance area; consequently, we spoke to that subject in the ordinance.

The time it has taken to develop this document has been lengthy. Sometimes it looked like it would never issue. We appreciate the input from all those persons who cared to participate in its construction. We believe we have reached a consensus regarding sanitation criteria for the retail food store industry which, if implemented, will result in better protection of the food supply at the retail level. The ordinance provides the minimum sanitary standards with which all retail food stores should be able to comply. In its reissued form, the document contains recommendations on some general provisions, food care, personnel, equipment and utensils, cleaning, sanitization and storage of equipment

and utensils, sanitary facilities and controls, construction and maintenance of physical facilities and compliance procedures. The true worth of the ordinance will be found in implementation of these recommendations through education and firm, equitable enforcement. We hope the model document will fulfill a current need. The ordinance will be included in the manual format like the present *Food Service Sanitation Manual*. The manual will contain reasons for the specific requirements, a short adoptive form of the ordinance, the recommended model ordinance, and other pertinent information. We expect approval by the end of 1979. Pre-publication copies should be available in early 1980 and printed copies from GPO in the spring of that year.

#### ACKNOWLEDGMENTS

Presented at the 66th Annual Meeting of International Association

of Milk, Food and Environmental Sanitarians, Orlando, Florida, August 12-16, 1979.

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## Update of the Fourteenth Edition of *Standard Methods for the Examination of Dairy Products*

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The Technical Committee preparing the Fifteenth Edition of *Standard Methods for the Examination of Dairy Products* desires to clarify and replace several paragraphs in the 14th edition. The Committee suggests that copies of these paragraphs be included in the appropriate sections or that notations be made in the current copies in the field. Reprints will be included with subsequent orders placed through the American Public Health Association. Single reprints for previously purchased copies may be ordered through the American Public Health Association.

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#### 4.7 Preparation of Phosphate-buffered Dilution Water and Testing for Toxicity

##### A. Stock phosphate buffer and magnesium chloride solutions:

1. Phosphate buffer: Dissolve 34 g of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) in 500 ml of microbiologically suitable (MS) water, adjust to pH 7.2 with 1 N NaOH solution, and make up to 1 liter with MS water. If desired, place in smaller vials, sterilize at 121 C for 15 min, tightly seal vials and store in refrigerator.

2. Magnesium chloride: To prepare stock solutions, measure 38 g of  $\text{MgCl}_2$  into a 1-liter volumetric flask and add MS water to make 1 liter of solution. Small, sealable vials may be filled with the stock solution, sterilized and stored as with the phosphate stock solutions.

##### B. Buffered dilution water:

To prepare dilution water, add 1.25 ml of stock phosphate buffer solution and 5 ml of stock  $\text{MgCl}_2$  solution to MS water and make up to 1 liter. Dispense as desired and autoclave at 121 C for 15 min. Addition of

magnesium chloride improves recovery of organisms with metabolic injury which may be induced by toxic properties in the dilution water.<sup>7,11,23</sup>

##### D. Dilution water toxicity test:

A screening test for dilution water toxicity can be conducted by preparing a dilution of the appropriate dairy food product to provide 100 to 300 colonies per plate when 1 ml is plated. The diluted sample(s) is then plated in duplicate at 0, 15, 30 and 45 min by the SPC<sub>2</sub> procedure (Chapter 5). Distinct trends toward lower counts among successively plated samples suggest probable toxicity. Decreases over 20% in 45 min are indicative of dilution water toxicity.

#### 4.12 References

23. Hartman, P. A. 1979. Modification of conventional methods for recovery of injured coliforms and salmonellae. *J. Food Prot.* 42:356-361.

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#### 5.11 Counting Colonies on Plates and Recording Results

Count plates promptly after the incubation period [5.10]. Record dilutions used and number of colonies on each plate counted or estimated. If impossible to count at once, store plates after the required incubation at 0-4.4 C for not more than 24 hr<sup>11</sup>, but avoid this as a routine practice. When counting colonies on plates, proceed according to directions given in 5.11(A). For each lot of samples, record the results of sterility tests on materials (dilution blanks, agar, etc.) used when pouring plates [5.9(B)] and the incubation temperature used.

##### A. Counting of colonies and recording counts:

1. Manual counting: Count colonies with the aid of magnification under uniform and properly controlled artificial illumination, using a tally [5.2(N)]. Routinely use a colony counter<sup>2</sup> equipped with a guide plate ruled in square centimeters [5.2(M)]. Plates should be examined in subdued light. Avoid mistaking particles of undissolved medium, sample or precipitated matter in plates for pinpoint colonies. Examine doubtful objects carefully, using higher magnification, where required, to distinguish colonies from foreign matter. Arrange schedules of laboratory analysts to prevent eye fatigue and the inaccuracies that inevitably result from eyestrain.

a. Normal (plates with 30-300 colonies or < 30

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<sup>8</sup>Department of Food Science and Technology, University of California, Davis, California 95616

colonies): Select spreader-free plate(s). Count all colonies, including those of pinpoint size on selected plate(s). Record dilution(s) used and total number of colonies counted.

b. Crowded plates (more than 300 colonies): Do not record counts on crowded plates from the highest dilution as too numerous to count (TNTC). If the number of colonies per plate exceeds 300, count colonies in those portions of the plate that are representative of colony distribution and calculate the Estimated Standard Plate Count from these counts. If there are fewer than 10 colonies per square centimeter, count colonies in 12 squares, selecting, if representative, six consecutive squares horizontally across the plate and six consecutive squares at right angles being careful not to count a square more than once. When there are more than 10 colonies per square centimeter, count colonies in four such representative portions. In both instances, multiply the average number found per square centimeter by the area of the plate used to determine the estimated number of colonies per plate. Each laboratory must determine the area in square centimeters of the plates in use.

c. Spreaders: Spreading colonies are usually of three distinct types. The first type is a chain of colonies, not too distinctly separated, that appears to be caused by disintegration of the bacterial clump. The second type is one that develops in the film of water between the agar and the bottom of the dish. The third type is one that forms in the film of water at the edge of or on the surface of the agar. If plates prepared from the sample have spreader growth such that (i) the area covered by the spreaders or the area covered by the spreader plus the repressed area exceeds 50% of the plate area, or (ii) the area of repressed growth caused by the spreaders exceeds 25% of the plate area, report the plate as "Spreader." When it is necessary to count plates containing spreaders not eliminated by (i) or (ii) above, count each of the three distinct spreader types as one source. If one or more chains appear to originate from separate sources, count each source as one colony. Do not count each individual growth in such chains as a separate colony. Types 2 and 3 usually result in distinct colonies and are counted as such. Combine the spreader count and the colony count to compute the plate count. Any laboratory with 5% of plates more than one-fourth covered by spreaders should take immediate steps to eliminate this trouble.

d. Laboratory accidents or bacterial growth inhibitor: When plate(s) from a sample are known to be contaminated or are otherwise unsatisfactory, record the plate(s) as Laboratory Accident (LA). If the test for inhibitory substances (Chapter 9) is positive for a sample, record the Standard Plate Count as "Growth Inhibitor" (GI). The analyst may be inclined to suspect the presence of inhibitory substances in the sample being examined when plates have no growth or have proportionally less growth in lower dilutions; such developments should not be interpreted as evidence of inhibition until the presence

of inhibitory substances has been confirmed.

2. Automated counting: Automated colony counters: Automated colony counters, when determined in individual laboratories to yield counts that 90% of the time are within 10% of those obtained manually, may be used for counting plates. When using colony counting instruments, exercise the following precautions:<sup>12</sup>

- align petri dish carefully on colony counter stage
- avoid "counting" stacking ribs or legs of plastic petri dishes
- do not count plates having unsmooth (rippled) agar surfaces
- avoid plates having food particles or air bubbles in the agar
- do not count plates having spreaders or extremely large surface colonies
- avoid scratched plates
- wipe fingerprints, and films off petri dish bottom before counting

### 5.12 Computing and Reporting Counts

To compute the Standard Plate Count, multiply the total number of colonies or the average number (if duplicate plates of the same dilution) per plate by the reciprocal of the dilution used.

When colonies on duplicate plates of consecutive dilutions are counted, compute the mean number of colonies for each dilution before averaging to report Standard Plate Count.

Avoid creating fictitious ideas of precision and accuracy when computing Standard Plate Counts, by reporting only the first two left hand digits. Round counts off to two significant figures only at the time of conversion to Standard Plate Count by raising the second digit to the next highest number only when the third digit from the left is 5, 6, 7, 8 or 9; use zeroes for each successive digit toward the right from the second digit.

#### A. Guidelines for computing counts

##### 1. Single plate dilutions:

a. One plate with 30-300 colonies: Use the dilution providing 30-300 colonies to compute the Standard Plate Count when the other Plate is outside of the 30-300 range or is excluded by spreaders or laboratory accident.

| Colonies/Dilution       | Example   |        |
|-------------------------|-----------|--------|
|                         | 1:100     | 1:1000 |
| <u>234</u> <sup>1</sup> | 28        | 23,000 |
| 340                     | <u>42</u> | 42,000 |
| Spr                     | <u>31</u> | 31,000 |
| <u>243</u>              | LA        | 24,000 |

b. Both plates with 30-300 colonies: When two dilutions yield counts of 30-300 colonies, average the two dilution counts obtained to compute the Standard Plate Count unless the count computed for the higher dilution is more than twice (> 2) the count computed for the lower dilution. In the latter case use the lower computed count as the Standard Plate Count.

<sup>1</sup>Underlined figures are used to calculate the SPC.

Example

| Colonies/Dilution |        | Count Ratio | Standard Plate Count |
|-------------------|--------|-------------|----------------------|
| 1:100             | 1:1000 |             |                      |
| 293               | 41     | 1.4         | 35,000               |
| 140               | 32     | 2.3         | 14,000               |

c. No plate with 30-300 colonies: When there is no plate with 30-300 colonies, use the plate having a count nearest 300 to compute the Estimated Standard Plate Count (ESPC).

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| 325               | 25     | 33,000 ESPC          |

d. Both plates with fewer than 30 colonies: When plates from both dilutions yield fewer than 30 colonies each, use the count from the lowest dilution to compute the Estimated Standard Plate Count.

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| 18                | 2      | 1,800 ESPC           |

e. Both plates with no colonies: When both dilutions yield plates with no colonies and inhibitory substances have not been found, use a count of less than (<) 1 times the lowest dilution plated to compute the Estimated Standard Plate Count.

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| 0                 | 0      | < 100 ESPC           |

f. Both plates have excessive spreader growth: When both dilutions yield plates with excessive spreader growth, spreader growth is reported for the Standard Plate Count.

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| Spr               | Spr    | Spr                  |

g. Both plates with more than an average of 100 colonies per square centimeter: When both dilutions yield plates with an average of greater than 100 colonies per square centimeter, estimate the Standard Plate Count as greater than (>) 100 times the highest dilution plated times the area of the plate.

The example below has an average count of 110 per square centimeter.

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| TNTC              | 7150*  | > 6,500,000* ESPC    |
| TNTC              | 6490** | > 5,900,000** ESPC   |

\*based on a plate area of 65 cm<sup>2</sup>, \*\*based on a plate area of 59 cm<sup>2</sup>

h. Both plates with more than 10 but less than 100 colonies per square centimeter: When both dilutions yield plates with greater than 10 but less than 100 colonies per square centimeter, use the estimated count of the highest dilution to compute the estimated Standard Plate Count. The example below has an average count of 15 per square centimeter when four squares are counted (5.11 A1.b).

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| TNTC              | 975*   | 980,000 ESPC         |

\*based on a plate area of 65 cm<sup>2</sup>

i. Both plates with more than 300 colonies but less than 10 per square centimeter: When both dilutions yield plates with more than 300 colonies but less than 10 per square centimeter, use the estimated count of the highest dilution to compute the estimated Standard Plate Count. The example below has an average count of 8 per square centimeter when 12 squares are counted (5.11 A.1.b).

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| TNTC              | 520*   | 520,000 ESPC         |

\*based on a plate area of 65 cm<sup>2</sup>

2. Duplicate plate dilutions:

a. Only one dilution yields plates with 30-300 colonies: When there is only one dilution in the 30-300 range, compute the mean for that dilution as the basis for the Standard Plate Count.

Example

| Colonies/Dilution |        | Standard Plate Count |
|-------------------|--------|----------------------|
| 1:100             | 1:1000 |                      |
| 175               | 16     | 19,000               |
| 208               | 17     |                      |

b. Both dilutions yield plates with 30-300 colonies: When both dilutions yield duplicate plates in the range of 30-300, average the mean count for each dilution as the basis for the Standard Plate Count unless the count computed for the higher dilution is more than twice the count computed for the lower dilution. In the latter case, use the lower computed count as the Standard Plate Count.

Example

| Colonies/Dilution |        | Count Ratio | Standard Plate Count |
|-------------------|--------|-------------|----------------------|
| 1:100             | 1:1000 |             |                      |
| 280               | 34     | 1.3         | 33,000               |
| 296               | 40     |             |                      |
| 138               | 42     | 2.4         | 15,000               |
| 162               | 30     |             |                      |

c. Neither dilution yields plates with 30-300 colonies: When neither dilution yields plates in the 30-300 range, use the mean count of the dilution nearest to 300 as the basis for estimating the Standard Plate Count.



| Example           |        |                      |
|-------------------|--------|----------------------|
| Colonies/Dilution |        | Standard Plate Count |
| 1:100             | 1:1000 |                      |
| <u>322</u>        | 23     | 33,000 ESPC          |
| <u>331</u>        | 29     |                      |

d. Both dilutions yield plates with fewer than 30 colonies: When duplicate plates of both dilutions yield fewer than 30 colonies, use the mean count of the duplicates of the lowest dilution to estimate the Standard Plate Count.

| Example           |        |                      |
|-------------------|--------|----------------------|
| Colonies/Dilution |        | Standard Plate Count |
| 1:100             | 1:1000 |                      |
| <u>18</u>         | 2      | 1,600 ESPC           |
| <u>14</u>         | 0      |                      |

e. Both dilutions yield plates with no colonies: When plates from both dilutions have no colonies, estimate the Standard Plate Count as less than (<) 1 times the lowest dilution.

| Example           |        |                      |
|-------------------|--------|----------------------|
| Colonies/Dilution |        | Standard Plate Count |
| 1:100             | 1:1000 |                      |
| <u>0</u>          | 0      | < 100 ESPC           |
| <u>0</u>          | 0      |                      |

f. Only one plate of one dilution contains 30-300 colonies: When one plate of one dilution contains 30-300 colonies and the duplicate contains more than 300 colonies, use both plates in computing the Standard Plate Count.

| Example           |        |                      |
|-------------------|--------|----------------------|
| Colonies/Dilution |        | Standard Plate Count |
| 1:100             | 1:1000 |                      |
| <u>322</u>        | 23     | 30,000               |
| <u>278</u>        | 29     |                      |

g. One plate of each dilution contains 30-300 colonies and the duplicate contains more than 300 or less than 30 colonies: When one plate of each dilution contains 30-300 colonies and the duplicate contains more than 300 or less than 30 colonies, use all four in computing the Standard Plate Count.

| Example           |           |                      |
|-------------------|-----------|----------------------|
| Colonies/Dilution |           | Standard Plate Count |
| 1:100             | 1:1000    |                      |
| <u>325</u>        | <u>27</u> | 32,000               |
| <u>290</u>        | <u>40</u> |                      |

h. Both plates of one dilution contain 30-300 colonies and only one duplicate of the other dilution contains 30-300 colonies: When both plates of one dilution contain 30-300 colonies and only one duplicate of the other dilution contains 30-300 colonies, use all four plates in computing the Standard Plate Count unless excluded by spreaders or laboratory accident.

| Example           |           |                      |
|-------------------|-----------|----------------------|
| Colonies/Dilution |           | Standard Plate Count |
| 1:100             | 1:1000    |                      |
| <u>278</u>        | <u>33</u> | 29,000               |
| <u>290</u>        | <u>28</u> |                      |
| <u>310</u>        | <u>33</u> | 33,000               |
| <u>290</u>        | <u>38</u> |                      |
| <u>274</u>        | <u>35</u> | 28,000               |
| <u>230</u>        | Spr       |                      |
| <u>269</u>        | <u>35</u> | 30,000               |
| <u>285</u>        | LA        |                      |

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Omit paragraph 8.33A.6 and the last footnote. All superscripts refer to the first footnote.

### 8.33 Wisconsin Mastitis Test

#### C. Procedure:

1. Rinse tubes and shake to remove excess water before use (or before calibration). Rinse syringe in water and well-mixed sample [5.6(B)]. Dispense 2 ml of well-mixed sample into each tube, running it down the side of the tube to avoid excessive foam.

2. Quickly add two 1-ml portions of reagent warmed to 35 C to each of the tubes in one rack to provide good initial mixing of milk and reagent. Deliver reagent below the surface of the milk (Fig. 8-3).

3. Promptly cap tubes with caps previously arranged right side up in front of the rack.

4. Start the mixing action about 30 sec after adding reagent to the first tube in the rack. Mix by holding tubes in nearly horizontal position and tilting them back and forth, permitting the liquid to run forward to the caps 10 times and return (Fig. 8-4). The 10 excursions should be made within 8-10 sec. During the forward tilting of the tubes, those samples most fluid should cover about half the cap (180°). During the backward tilt, the butts of the tubes should move down through an arc of about 10-15 mm. Avoid vigorous agitation. The temperature of the milk-reagent mixture at the time of inverting must be  $24 \pm 2$  C ( $75 \pm 4$  F).

Note 1: The following should be helpful in establishing uniform technique: To guide the tilting movement, place the back of a WMT rack on top of a rod 5 to 6 mm in diameter, or a 1-ml milk pipet, lying on the laboratory table. The rod acts as a rocker and should reduce the tendency to tilt tubes too far backward. Minimize touching the table top with the rack during forward and backward tilts.

5. Invert the rack within 30 sec after the mixing action. Before inverting, hold the tubes in a horizontal position while waiting for the sweep second hand on the timer to reach a convenient starting point. Then invert the rack rapidly but smoothly and hold it in a vertical position through a 15-sec outflow (Fig. 8-5). Return the tubes to an upright position, remove the caps and let stand for at least 1 min to allow to drain down before

reading.

6. Use the measuring square [8.33(A.10)] to measure the fluid column remaining in each tube (Fig. 8-6). Measure to the top of the meniscus and record readings in millimeters.

Note 2: Clean caps by placing them in a small container of warm water and shaking them in two or three changes of water. Rinse the tubes two or three times in warm tap water (not over 45 C (113 F)) after each use. Remove water by shaking the rack before each use.

#### D. Precautions

1. For calibration of caps, use the special WMT nozzle gauge [8.33 (A.13)] or equivalent. Correct size of the orifice is indicated when the end of the WMT nozzle gauge wire can be inserted into the orifice 1.5 to 2.5 mm without pressure.

2. With continuous use, WMT tubes change shape. Frequent checking of calibration is therefore necessary. WMT tubes have correct inside dimensions when the combined height of the column of 2 ml of milk plus 2 ml of reagent reaches 37 mm. Approved glass pipets

must be used to check calibration. Tubes not meeting this standard must be discarded.

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#### 18.4 Scharer Rapid Phosphatase Test

##### A. Equipment:

8. Color standards: Prepare phenol standards [18.4 (C.3)] to contain 1, 2 and 5  $\mu\text{g}$  of phenol per 5 ml of solution. Alternatively, commercial standards are available. (One source is Applied Research Institute, 40 Brighton Ave., Perth Amboy, NJ 08861.)

##### E. Procedure:

6. Remove tubes from the water bath, cool in an ice water bath, add 3 ml of neutralized cold n-butyl alcohol, restopper, and extract indophenol blue by gently inverting the tubes four times through a half circle. (Table about 1 sec to invert tubes, pause about 1 sec, take another 1 sec to return tubes upright, pause 1 sec and then repeat.) Lay tubes on their sides on a flat surface for 2 min to permit separation of the butyl alcohol, then repeat the mixing and separation steps.

### Another Request for Research Proposals

The Technical Committee that is preparing the 15th edition of *Standard Methods for the Examination of Dairy Products* would like to add the project given below to those that appeared in the March, 1980 issue of this *Journal*. Please refer to the March issue for details on preparing a research proposal.

#### *Compositing of Samples for Phosphatase Testing*

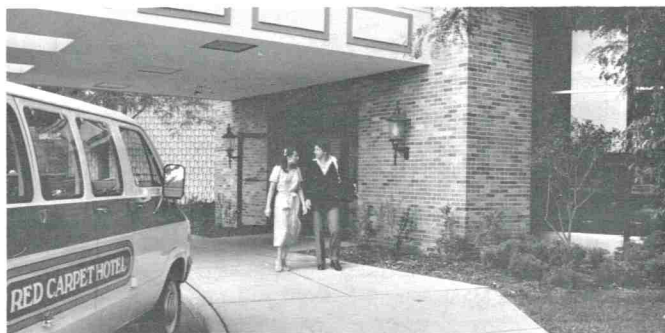
Current Procedures require testing of individual pasteurized milk samples. Failure to properly pasteurize milk normally leads to significant phenol equivalent values. Compositing of samples may be possible yielding significant analytical time savings when large sample volumes

are tested. The project should consider minimal phenol equivalent values detectable when samples are composited as well as the effect of

compositing on the determination of microbial and reactivated phosphatase. For further information contact Dr. H. M. Wehr, Oregon Department of Agriculture, 635 Capitol Street N.E., Salem, Oregon 97310. Telephone: (503-378-3793).

# An Invitation from America's Dairyland:

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| Registration      | \$20.00                                                                         | \$ 8.00                 | No chg.        | \$25.00                                                       | \$11.00                 | No chg.        | \$30.00          |
| Banquet           | 15.00                                                                           | 15.00                   | \$15.00        | 17.50                                                         | 17.50                   | \$17.50        | 22.50            |
| Gemütlichkeit     | 2.00                                                                            | 2.00                    | 2.00           | 3.00                                                          | 3.00                    | 3.00           | 5.00             |
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| <b>Total</b>      | <b>\$37.00</b>                                                                  | <b>\$25.00</b>          | <b>\$17.00</b> | <b>\$45.50</b>                                                | <b>\$31.50</b>          | <b>\$20.50</b> | <b>\$57.00</b>   |

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## Coming Events/News and Events

April 21-25--TRAINING THE TRAINER, Seminar. Manhattan, KS. Sponsored by American Institute of Baking. Conducted by Adult and Occupational Education Dept. of KSU College of Education, along with AIB staff. Fee: \$450, space limited to 36 participants. Contact: Dr. Darrell Brensing, AIB, 1213 Bakers Way, Manhattan, KS 66502, 913-537-4750.

April 22, 24--MINNESOTA SANITARIANS ASSOCIATION, Outstate Spring Educational Meetings. April 22 - Albert Lea, MN; April 24 - Alexandria, MN. Contact: Ed Kaeder, Mid America Dairymen, Inc., 2424 Territorial Road, St. Paul, MN 55114, 612-646-2854.

April 28-30--BAKING PRODUCTION TECHNOLOGY, Regional Seminar, Atlanta, GA. Co-sponsored by American Institute of Baking and Southern Bakers Assoc. Fee: \$300. Contact: Dr. Darrell Brensing, AIB, 1213 Bakers Way, Manhattan, KS 66502, 913-537-4750.

April 29--1980 PROFESSIONAL EDUCATIONAL SEMINAR, "Achieving Cost-Effective Quality Foodservice in Institutional and Health Care Facilities." Purdue University, West Lafayette, IN. Sponsored by the Restaurant, Hotel, and Institutional Management Institute (RHIMI) at Purdue University. Certification and credit approved by American Dietetic Association; Hospital, Institutional and Educational Food Service Society; continuing education units from Purdue University. For more information, contact: John Palmer, Asst. Director, RHIMI, Purdue University, Stewart Center, West Lafayette, IN 47907, 317-749-2367.

April 30--USE AND ABUSE OF FOOD SUBSTANCES---TECHNOLOGICAL AND HEALTH IMPLICATIONS. Pick Congress Hotel, Chicago, IL. Sponsored by Illinois State Medical Society, Chicago Nutrition Association and Chicago Section---Institute of Food Technologists. Contact: Therese Mondeika, American Medical Association, 312-751-6524, or CNA-IFT-ISMS Symposium, c/o Lucy Morrow, Box 502, Glenview, IL 60025.

May 13-15--SOUTH DAKOTA ENVIRONMENTAL HEALTH ASSOCIATION, ANNUAL EDUCATION CONFERENCE. Huron, SD. Contact: Cathy Meyer, Box 903, Mitchell, SD 57301, 605-996-8231 or 605-996-4131.

May 15-16--ENVIRONMENTAL LAWS & REGULATIONS COURSE. Hotel Washington, Washington, D.C. Two-day course on latest regulatory developments. Contact: Government Institutes, PO Box 5918, Washington, DC 20014, 301-656-1090.

May 18-20--MIDWEST DAIRY PRODUCTS ASSOCIATION, Annual Convention. Pheasant Run Lodge, St. Charles, IL. Contact: MDPA, 5610 Crawfordsville Road, Suite 1104, Indianapolis, IN 46224, 317-243-9341.

June 2-4--USE OF ANIMAL PRODUCTS IN HUMAN NUTRITION. Iowa State University. Three-day symposium sponsored by the Nutrition Foundation, Inc. and Iowa State University Nutritional Sciences Council. Contact: Dr. Donald C. Beitz, 313 Kildee Hall, Iowa State University, Ames, IA 50011, 515-294-2063.

June 2-5--PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE. Keller Conference Center, The Pennsylvania State University, University Park, PA 16802. Contact: William Killough, RD 1, Box 393, Conestoga, PA 17516.

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

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

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

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

July 6-11--XI INTERNATIONAL SYMPOSIUM ON PSYCHROTROPHIC MICROORGANISMS IN SPOILAGE AND PATHOGENICITY. Aalborg Universitetscenter, Sohngaardsholmsvej 57, Aalborg, Denmark. Sponsored by International Association of Microbiological Societies, Committee on Food

Microbiology and Hygiene. Contact: Secretariat, IAMS, XI International Symposium, Institute of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870, Copenhagen V, Denmark.

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

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

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

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

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

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

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

## NAMA List Ready

The National Automatic Merchandising Association (NAMA) "Listing of Letters of Compliance," may be obtained from NAMA headquarters.

The list contains vending machine manufacturers and models which have been evaluated and certified under the NAMA Machine Evaluation program, conducted at Indiana and Michigan State Universities.

Copies of the listing are available free to regulatory, military, and educational institutions. Contact: NAMA, 7 S. Dearborn St., Chicago, IL 60683, 312-346-0370.

## Anderson is Honored

Anderson Instrument Company recently received the Food Industry Suppliers Association award as outstanding manufacturer of 1978. The award cited Anderson for "its distributor support programs." Anderson markets temperature and pressure instrumentation for sanitary fluid processing through a nationwide network of distributors.

Don Hofius, president of the Food Industry Suppliers Association, presented the award to Robert C. Anderson, president of Anderson Instrument Co., at FISA's Annual Distributor/Manufacturers Conference. The Association consists of distributors of equipment and supplies to food processors in the United States and Canada.

## Journal Seeks Reviewers

The *Journal of Food Protection* is looking for persons who'd like to write book reviews for publication in the *Journal*.

We receive a number of books from publishers which are worthy of review. Reviewers may keep the books they review.

If you're interested in writing reviews, please send your name and address, along with the subject matter you're interested in covering. Send the information to: Jan Richards, Assoc. Managing Editor, *Journal of Food Protection*, PO Box 701, Ames, IA 50010.

## Crumbine Award Competition Open

Entries are now being accepted for the 1980 Samuel J. Crumbine Consumer Protection Award. It has honored local government public health agencies since 1955 for "excellence in programs of food and beverage sanitation" in public eating and drinking establishments.

The competition is open to local government units in cities, counties, districts, towns, and townships which are responsible for the design and execution of sanitation and safety programs to protect consumers in public eating and drinking places.

The criteria on which an independent panel of jurors will judge

entries include program improvement, innovative and effective use of evaluation methods, effectiveness of planning and management, and excellence of information and education activities.

Deadline for the 1980 award entries is June 30, 1980.

Presentation of the award will be made at the annual meeting of the American Public Health Association in Detroit, Michigan, October 19-23, 1980.

Applications may be obtained by writing to the award sponsor, the Single Service Institute, Inc., 1025 Connecticut Ave., N.W., Washington D.C. 20036.

## Sherman Award Details Near Completion

Plans are being completed for the Norbert F. Sherman Award, to be presented for the first time at the 1980 IAMFES Annual Meeting. The award, which will be funded by the National Institute for the Food-service Industry (NIFI), will recognize the top foodservice food protection article to be printed in a year's volume of the *Journal of Food Protection*.

Judges have been selected for the

award and they include: Dee Clingman, Chairman, Director of Quality Control, Red Lobster Inns of America; K. J. Baker, Senior Food Consultant, Division of Retail Food Protection, FDA; Charles Felix, Editor, *Environment News Digest*, Single Service Institute; and Dr. Dennis Westhoff, Professor, Dept. of Dairy Science, University of Maryland. The NIFI liaison is Patricia Dames, Director of Special Projects, NIFI.

## FDA Catalogs Highlight Courses, Visual Aids

Two catalogs available from the FDA outline courses and visual aids available through the agency.

"FDA Training Bulletin," offers information on FDA training courses in food protection related areas, as well as a list of training aids and films available.

Among the courses available at different locations around the country are, "Milk Pasteurization Controls and Tests," "Grade A Pasteurized Milk and Milk Products," "Microbiological Aspects of Food Processing," "Current Concepts in Food Protection," and "Legal Aspects of Enforcement."

"Visual Aids on Food Service and

Processing," provides a list of food service, protection and processing materials available from many sources, including IAMFES, the American Dietetic Association, American Frozen Food Institute, Single Service Institute, and National Sanitation Foundation.

For more information on the courses or to order the catalogs, contact: U.S. Dept. of Health, Education and Welfare, Public Health Service, Food and Drug Administration, Executive Director of Regional Operations, Division of Federal-State Relations, State Training Branch, 550 Main Street, FOB, Room 8002, Cincinnati, OH 45202.

## News and Events

The following reports are among those given at the last Annual Meeting of IAMFES. The *Journal* policy is to print these as space allows.

### Annual Report BISSC Committee

This Committee met with the Baking Industry Sanitation Standards Committee (BISSC) at both the 1978 fall meeting in San Francisco, California and the 1979 winter meeting in Chicago, Illinois.

At the San Francisco meeting a new BISSC Standard was added, "Installation of Bakery Equipment, #37."

This standard originally part of "Basic Criteria," has been expanded and is available for distribution. The "Basic Criteria" has been reprinted to reflect the change.

Paul Laughlin of NABISCO, Inc. retired as chairman of BISSC and has been replaced by William E. Pieper of the American Society of Bakery Engineers, a long-time proponent of public health and sanitation and the principles of the IAMFES.

I was requested to, and will continue to, serve on the BISSC Certification Board, providing IAMFES with representation and input to that particular function of BISSC.

At the winter meeting in Chicago, Illinois, the BISSC Committee approved one standard and referred three other proposed standards to task committees until the fall meeting in Kansas City.

The standard for "Grinders, Pulverizers, Comminuters, Food Choppers and Breakers" was changed in name to "Particle Size Reduction Equipment" and approved for 60-day circulation for final approval, prior to publication.

The Standard for "Sugar Wafers and Cones, and Pretzels" requires expansion and additional input from members of both users and manufacturers and was returned to task committee for action at the Fall meeting.

The Standard for "Dough Forming Equipment" was not completed and was returned to task committee for refinement and discussion at the Fall Meeting.

The Standard "Packaging Equipment" requires further refinement and discussion to avoid conflict with existing Standard #13 for "Bread, Cake and Roll Slicing, Wrapping and Bagging Machines." This was referred back to task committee for action at the Fall Meeting.

In addition to the agenda of administration and financial matters routinely handled by the "Certification Board" several other items of importance were discussed.

One concerned my referral to the Board of the Federal Trade Commission (FTC) mandatory standards proposed for the regulation of committee and organizations engaged in the formulation of standards for all types of industries, which includes BISSC.

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### IAMFES Foodservice *Journal* Committee

The committee has accomplished the following two tasks during the past year -

1. As the result of outstanding effort by K. J. Baker, an index or bibliography of foodservice articles which have appeared in the *Journal of Food Protection* over the past five years has been developed. The index was published in the February, 1980 issue of the *Journal of Food Protection*. Baker has provided two addendums to the original index to incorporate current articles. A similar index was also developed by Baker for the *Journal of Environmental Health*.
2. The committee has been successful in establishing a new award to be given by the National Institute for the Foodservice Industry (NIFI). This new award is based on the following guidelines -
  - A. For three consecutive years, commencing with the 1980 IAMFES Annual Meeting, NIFI will present an award for an outstanding article in foodservice food protection, which has been published in the *Journal of Food Protection*, during a one-year period.
  - B. Where an article recipient has co-authorship, the senior or primary author will receive the cash award.
  - C. The Chairman of the Journal Foodservice Committee shall appoint a panel of judges, confirmed by the editor, of *JFP* to:
    - (1) Establish guidelines for the selection of articles qualifying for the award.
    - (2) Establish procedures for evaluation of articles and their subsequent review.
    - (3) Select the winning article.
  - D. If in any annual award year insufficient articles are available for qualification or if the panel of judges do not select a winning article, no award shall be given for that year.
  - E. The award shall consist of a recognition plaque and \$200.00 cash.
  - F. A representative of NIFI shall present the award during the IAMFES Annual Meeting at a date and time selected by the IAMFES Executive Board.
  - G. After the IAMFES Annual Meeting in 1982, NIFI and IAMFES will confer on the success of the award program and make recommendations regarding continuance if so warranted.

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### Report of IAMFES Representative to IDF

Progress is finally being made in our efforts to have the U.S. become a member of the International Dairy Federation (IDF).

For several months, small groups have been meeting in an attempt to develop the framework for the formation of an interim US/IDF Committee, the first step toward official U.S. involvement.

A meeting was held on June 5, with representatives from ADAMI/WPI, UDIA, IAMFES and several industry members to prepare a draft for a functioning group for the following purposes.

1. Establish proposed objectives of the committee
2. Prepare an organizational program
3. Solicit a broad-based membership
4. Educate members and non-members on IDF.
5. Assess the need and desire for a permanent U.S. National Committee.

As a result, a "Proposed Functioning of an Interim U.S. National Committee of the International Dairy Federation" was prepared and distributed to a number of organizations.

Another well-attended meeting was held during the ADSA Annual Meeting in Logan, Utah on June 25.

Dr. Kenneth Savage, president of IDF, attended and reviewed the background and function of the IDF and a proposal that the IDF accept the "interim" committee as a member for a two-year trial period, without payment of national committee dues.

The response from these introductory meetings has been positive. It was decided to initiate a call for membership at a meeting to be held at UDIA headquarters, Rosemont, Illinois on July 20, 1979. Along with Dr. Savage, Dr. Hans Kay, President of the IDF Commission of Studies would attend that meeting.

Most of the organizations listed attended and temporary officers were chosen to guide the organization until a permanent group is formed as follows:

Chairman - Fred J. Greiner (Dairy and Food Industries Supply Association)  
Treasurer - Gregory M. Farnham (Dairyland Food Laboratories)  
Secretary - Harold Wainess (IAMFES)

The Secretary was instructed to send a letter to Pierre Staal, Secretary-General of the IDF, indicating that the interim National Committee has been formed and requesting formal recognition of the Committee by the IDF at their annual meeting in Montreux, Switzerland, September 9-14.

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**BISSC Report**, *con't from p. 332*

Since I have been closely associated with the 3A Sanitary Standards Committee for many years, a recommendation was made that I be appointed as this group's liaison between the 3A Committee and BISSC which would avoid conflict between standards for similar equipment used in both the bakery and dairy industries. This recommendation will be referred to the BISSC Executive Board.

At present there are BISSC Standards covering thirty-seven (37) categories of Baking Equipment. Seventy (70) companies have been authorization certificates for equipment manufactured in compliance with the stand-

ards. BISSC information booklets as well as all BISSC Standards are available and we urge all sanitarians to acquire a set of these standards and adopt them as guidelines and subscribe to the principles represented by the BISSC Standards and Criteria.

Copies may be obtained by writing to Ray Walter, Executive Secretary, BISSC, 521 Fifth Ave., New York, N.Y. 10017.

Respectfully submitted,

Martyn A. Ronge  
Chairman Illinois Assoc.  
Harold Wainess & Assoc.  
464 Central Ave.  
Northfield, Illinois 60093

Jerome A. Mithen, Jr.  
(Ill. Assoc.)  
P.O. Box 1148  
Manhattan, Kansas 66502

Phillip E. Winters (Ohio Assoc.)  
5446 Karen Ave.  
Cincinnati, Ohio 45211

Tom Rolfe (N.Y. Assoc.)  
Dir. of Sanitation  
Continental Baking Co.  
P.O. Box 731  
Rye, New York 10580

**Foodservice Journal Report**, *con't from p. 332***Committee Meetings**

Committee functions were carried out mostly by mail and telephone communication. A committee meeting was held on August 13, 1979. Those in attendance were: Dee Clingman, Patricia Franks, K. J. Baker, and Elmer Marth. At this meeting the following projects were discussed for the upcoming year:

1. Finalizing the new NIFI award procedure.
2. Developing a system to solicit articles in the area of foodservice.
3. Promoting research and encouraging submission of manuscripts in the following areas:
  - Day care feeding centers
  - Meals on wheels
  - Role of vegetables in foodborne illness
  - Nutrition in foodservice
  - Bar sanitation
  - Local health department foodservice projects or education programs
  - Raw product quality - source sanitation
  - Industry quality assurance programs

4. Committee members will be contacted to ascertain their availability to serve for 1979-80. Where necessary, new committee members will be added.

Respectfully submitted,

C. Dee Clingman, Chairman

**Committee Members**

K. J. Baker, R.S.  
Senior Food Consultant  
Food & Drug Administration  
Prof. Ruth S. Dickie  
Department of Continuing Medical Education  
University of Wisconsin  
Dorothy Ellis, R.P. Dt.  
Food Technology Division  
George Brown College  
Dave Hartley, Director  
Public Health  
National Automatic Merchandising Assoc.  
Earl Helmreich  
Food Protection Unit  
Ohio Department of Health  
Fred Mitchell, Chief  
Hotels, Resorts & Restaurants  
Minnesota Department of Health

Roy Moser  
Extension Food Technologist, Professor  
University of Hawaii

Robert Pickenpack  
Director, Product Safety  
General Mills, Inc.

Barry Preswick  
Quality Assurance Supervisor  
McDonald's Corporation  
Thomas Schafer, Director  
Quality Control & Sanitation  
Pizza Hut, Inc.

Dr. Oscar Snyder, Associate Professor  
Department of Food Science & Nutrition  
University of Minnesota  
Gail Terreri, Microbiologist  
Arthur Treacher's Fish & Chips  
Dr. Nan Unklesbay, Assistant Professor  
Department of Food Science & Nutrition  
University of Missouri

James C. White  
Professor, Hotel Administration  
Cornell University

Patricia Dames  
Director, Special Projects  
National Institute for the Foodservice Industry

**IDF Report**, *con't from p. 332*

This year, due to the short time involved and conflict with meetings in the US, there will only be a small delegation at the IDF meeting.

Dr. Kay reviewed the operations of the National Committees, the commissions and the Groups of Experts. At this point anyone appointed by the Interim Committee is privileged to attend the meetings and comment on various reports as observers. During the formation of a permanent commission, the U.S. Commission has offered to work with various groups of experts on a correspondence basis.

For the Montreux meeting (tentatively) the following represent the USA:

Commission A, Hygiene and Quality of Milk - Dr. W. B. Schultze (National Mastitis Council)

Commission B, Technology and Engineering - Harold Wainess (IAMFES)

Commission C, Economics, Marketing and Management - no official representative

Commission D, Legislation, Standards of Identity, Classification,

Terminology - (To be chosen)

Commission E, Analytical Standards, Laboratory Techniques

1. Chemistry - Dr. William Horowitz/  
Dr. R. Weik (AOAC)

2. Microbiology - Dr. R. B. Read  
Commission F, Science and Education - Representative to be appointed by ADSA  
Gregory Farnham might also be able to attend the sessions.

Dr. Savage pointed out that when Canada joined the organization a number of years ago, their problems were similar to ours. He suggested that a meeting be held with the Canadian representatives. He also suggested that we attempt to hold a joint meeting with the Canadian IDF group during their annual meeting in Canada, which will take place after the Montreux meeting.

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# IAMFES AFFILIATE OFFICERS

Note to officers: Please check for accuracy of your listing. If changes are necessary, send them to: Jan Richards, *Journal of Food Protection*, PO Box 701, Ames, IA 50010.

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**IDF Report, con't from p. 333**

The Secretary will also attend the meeting of the Commission of Studies (CE) and obtain a draft of the report for distribution in the U.S. This is the Group that receives all recommendations made by the various Commissions during each Annual Meeting and determines which will be accepted, what material will be published and what special seminars will be held.

After the Montreux meeting, the Secretary was asked to prepare a list of all of the active group of experts.

In order to further develop enthusiasm within the U.S. dairy industry, an informational program has to be prepared to describe how the U.S. will benefit from IDF membership. This will be followed by a recruitment program to obtain, as soon as possible, and perhaps on a regional basis, greater U.S. participation in the interim committee.

A release will be sent to all the dairy publications, describing the present activities, the importance of the involvement of the U.S. and proposed functioning, both on an interim and permanent basis. This release would also ask all those who are interested in becoming involved to notify the interim National Committee. All inquiries relative to participation in the organization should be made through the Secretary. If at all possible, a mailing with similar information should also be sent to the industry before September 1. Dr. Kay requested that a copy of our public release be sent to him for distribution to the various national committees.

It was recommended that the officers hold a meeting on August 8 to review the minutes and that the minutes then be distributed to all those present at the July 20 meeting.

Another meeting of the Interim Committee

will be held during the week of August 27. At that time, an active steering committee will be appointed.

After the Montreux meetings, there will be another meeting of the entire group or the steering committee to determine how to structure the temporary officers in the U.S. It could be held October 24 during the Chemical Week Symposium sponsored by AOAC, IDF, and ISO in Washington, D.C. Dr. Kay will be there and suggested that time be allotted to discuss progress and further steps to be taken.

The document entitled "Proposed Functioning of an Interim U.S. National Committee to the International Dairy Federation" was reviewed and it was recommended that Paragraph 4b be changed to read "Members shall pay an initial fee of \$100.00." The

con't p. 320

## Positions Available

### Food Process Scientist

Food Process Scientist-Lecturer or Assistant Professor, Dept. of Biochemistry, Memorial University of Newfoundland.

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### Extension Food Technologist

The Department of Food Technology, Iowa State University, Ames, IA, invites applications for the position of Extension Food Technologist, with accompanying tenure track, 12-month appointment as Assistant or Associate Professor of Food Technology. Position is 100% Extension with requirement to develop and conduct an extension program for the Iowa food processing industry. M.S. or Ph.D. required in food science and technology and with above average communications skill. Food processing experience preferred. Minimum salary of \$21,000. Applications accepted until June 15, 1980 or until candidate is selected. Send resume, transcripts, and 3 letters of recommendation to: Dr. W. S. LaGrange, Dept. of Food Technology, Iowa State University, Ames, IA 50011.

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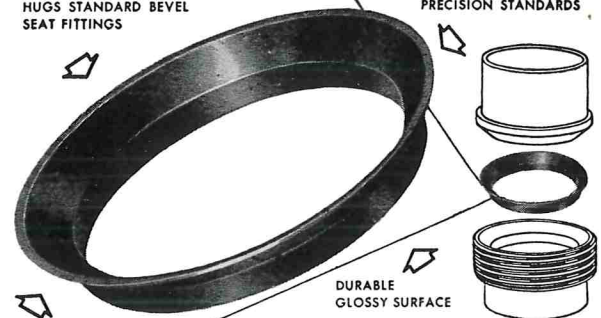
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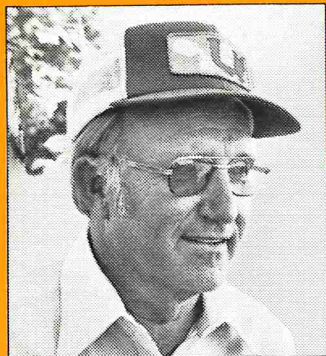
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# ***SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY***



**Ed Kaeder, Milk Quality Fieldman**

*Mr. Kaeder is Field Supervisor, member services for Mid-America Dairymen Inc., Northern Division—a regional dairy cooperative. He was born and raised on a dairy farm and graduated from the University of Minnesota in dairy husbandry in 1944. He worked as an extension fieldman before becoming involved in milk quality control more than 30 years ago.*

"The anatomy of a good fieldman involves being familiar with milk products from the cow all the way through to the customer's table. Since fieldmen work with dairy farmers whose livelihood is production of milk, it is only right to note that milk is probably the most regulated agricultural product in America today. In many cases, government regulates the flow of products, sets minimum prices, and makes rules under which we operate. Basically the fieldman functions as the milk plant's personal contact with its members and is a goodwill ambassador.

"There are many reasons a fieldman will contact members, but the main reason is usually quality. Other calls may be about Grade A requirements, herd health, flavor control, farm building plans, milking equipment installations, arrangements for purchase of equipment, member relations dealing with problems and complaints.

## **Knowledge And Testing Important**

"There are many tools for the fieldman to use in assisting the producer of high quality milk. Among these are the numerous tests done by every dairy plant. The fieldman must be familiar with these tests: raw count, pasteurized count, cell counts and keeping quality.

"Care of milking equipment is important as well because no other piece of equipment on the farm will get an unsuspecting or careless operator into trouble faster than a faulty or poorly operated milking machine. Lack of proper sanitation will increase bacteria counts, and poor operation will contribute to poor udder health.

"The fieldman must be thoroughly familiar with cleaning and sanitizing compounds and their various uses. He must be in a position to help the dairy producer set up a cleaning program to be followed after each milking, and assure there are no shortcuts in the procedure.

"Requirements for the production of Grade A milk are spelled out in detail in the Grade A Pasteurized Milk

Ordinance (PMO), a publication of the FDA and U.S. Public Health Service. We must be able to translate and interpret these requirements for members so they can and will maintain a Grade A status.

"We are involved in herd health with dairymen too, especially in areas relating to causes and prevention of mastitis. We must be able to make use of tools and testing devices because they can tell a great deal and be an aid in educating the dairyman.

"The problem of antibiotics in the milk supply seems to require more and more of our time and attention. Processors can't make cheese and cultured products from antibiotic milk because desirable bacteria will not grow, and any drug residues are unacceptable in milk.

"Working closely with milk haulers is another important aspect for the fieldman since most haulers have the closest and most frequent contact with members. A conscientious hauler is invaluable to us in performing effective fieldwork for the dairyman.

"Today dairy farmers account for only about 0.1% of the population. The distance between active dairy farms becomes longer and, in the interest of energy conservation, and making the most of the dairyman's time, a daily plan of farm calls in a given area is outlined so a minimum amount of time and miles will be expended in driving.

"In summary, I will say that the anatomy of a good fieldman is public relations. In this analogy public relations means selling yourself by being interested, optimistic and enthusiastic about the job at hand, and being informed on all aspects of milk, the industry and your organization."



## **SURGE**