Fate of *Salmonella typhimurium* and *Staphylococcus aureus* in Meat Salads Prepared with Mayonnaise

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**ABSTRACT**

*Staphylococcus aureus* and *Salmonella typhimurium* were tested for their ability to survive and to multiply in meat salads prepared with different concentrations of mayonnaise and held at 4, 22, and 32°C. When mayonnaise was added to meat salads in amounts recommended by recipes from a reputable cookbook, it inactivated a substantial portion of the initial population of both *S. aureus* (30-60%) and *S. typhimurium* (20-25%). Salads that were refrigerated at 4°C for 24 h evidenced very little growth of either organism whether mayonnaise was present or not. Storing salads at 22 or 32°C for 5 h resulted in <1.0 log_{10} increase of either organism with the greatest increase occurring in salads containing no mayonnaise. Mayonnaise retarded but did not prevent the growth of *S. aureus* or *S. typhimurium* in salads stored at 22 or 32°C for 24 h. Increasing the concentration of mayonnaise in salads increased the degree to which growth of these organisms was delayed. Contrary to popular belief, the presence of mayonnaise in meat salads tends to retard rather than enhance growth of food-borne pathogens. However, addition of mayonnaise should not be considered a substitute for refrigeration for preserving meat salads from the growth of food-borne pathogens.

A common misconception that has persisted for many years is that adding mayonnaise to cooked meats, such as chicken or ham, will encourage the growth of food-borne disease agents and subsequently increase the risk of food poisoning if the food is not properly refrigerated. Many individuals do not realize the commercially prepared mayonnaise is an unfavorable medium for growth and survival of most bacteria (4, 7, 10, 14).

Mayonnaise that is prepared commercially in the United States is a standardized food whose composition is specified by the U.S. Food and Drug Administration (13). Vegetable oil comprises at least 65% of the finished product while the remaining 35% consists of vinegar, lemon, and/or lime juice, and egg yolk or whole eggs. If desired, manufacturers may also add salt, sugar, monosodium glutamate, spices, citric and/or malic acid, a sequestrant, and crystallization inhibitors. Once these ingredients are emulsified, the final product has a pH of 3.6 to 4.0 and a water activity of about 0.925 (10). If salt is added, the aqueous phase contains 9.0 to 11% sodium chloride (10). This is a hostile environment for harmful bacteria. Many studies have shown that *Salmonella* sp. and *Staphylococcus aureus* cannot survive but actually die off when they are present in mayonnaise (4, 7, 10, 14).

Although much is known about the fate of food-borne pathogens in mayonnaise, few data are available which identify what happens to *Salmonella* sp. or *S. aureus* in meat salads that have been prepared with mayonnaise and stored at abusive temperatures. Hence, the purpose of this study was to determine how these organisms would respond in meat salads that contained different concentrations of mayonnaise and were held at temperatures representing refrigeration, room temperature, and an extreme temperature that salads may receive during a hot summer day when enroute to and served at a picnic.

**MATERIALS AND METHODS**

**Design of experiment**

Chicken and ham salads were each prepared with three different concentrations of mayonnaise, i.e., none, one-half, and the actual amount recommended by recipes obtained from a reputable cookbook. Each preparation of salad was inoculated with *S. typhimurium* or *S. aureus* or left uninoculated. The uninoculated salads served as controls to indicate if any *Salmonella* spp. or *S. aureus* were present in the salads as contaminants before inoculation or during the course of the experiment. Each of the inoculated and uninoculated salad preparations were divided into seven equal portions of which one portion each was analyzed immediately after preparation and after incubation at 4, 22, or 32°C for 5 or 24 h.

**Preparation of inocula**

*Salmonella typhimurium*, obtained from E. H. Marth (Dept. of Food Science, University of Wisconsin-Madison), and *S. aureus* strains 196-E and S-6, obtained from M. S. Bergdoll (Food Research Institute, University of Wisconsin-Madison), were used for these studies. Each
culture was maintained on Nutrient agar (Difco) slants at 4°C. Inocula were prepared by transferring a loopful of each culture from Nutrient agar to 10 ml of Nutrient broth (Difco) and incubating at 37°C for 16 h. Cells were removed by centrifugation (10,000 × g, 10 min), washed three times with 0.1% peptone-water, and resuspended to a concentration that would result in 1.5 × 10³ cells/g of salad. One ml of bacterial suspension was added to 250 g of salad. Approximately equal numbers of cells of the two strains of S. aureus were combined and inoculated into each salad.

Preparation and inoculation of salads

With slight modifications, chicken and ham salads were prepared according to recipes outlined in the New Pillsbury Family Cookbook (The Pillsbury Company, Minneapolis, MN). The proportions of ingredients used to formulate the chicken salad included 3 cups (370 g) of cubed, cooked chicken, 2 stalks (125 g) of chopped celery, and 0.1/4 (60 g), or 1/2 (120 g) cup of commercial mayonnaise. Omitted from the original recipe were 2 tablespoons of lemon juice. The ham salad consisted of 3 cups (450 g) of cubed, cooked ham, 1/2 cup (80 g) of pickle relish, 2 (130 g) chopped, hard-boiled eggs, 1 stalk (62 g) of chopped celery, and 0.1/4 or 1/2 cup of mayonnaise. A dash of pepper and 1/2 teaspoon of dry mustard were omitted from the original recipe. The greatest amount of mayonnaise used, i.e., 1/2 cup for both chicken and ham salad, was the amount actually specified by the Pillsbury recipes. To prepare enough salad for each study, eleven times the amount of the recipe for chicken salad and eight times that of ham salad were prepared for each concentration of mayonnaise evaluated.

For more uniform distribution of the ingredients, the chicken, ham and hard-boiled eggs were individually ground through a template of Whirlpak bags. KS-A, uninoculated to serve as a control. Salads were inoculated by dropwise addition of the bacterial suspension as the salald was being mixed continuously. Each treatment of salad was then subdivided into seven equal portions of which not less than 250 g were added individually to Whirlpak bags.

Enumeration of bacteria

Based on the observations of earlier investigators who have shown that commercially prepared salads generally contain less than 10 Enterobacteriaceae per g and very low initial counts of S. aureus (5) and on the fact that extra precautions were taken to maintain aseptic conditions during the preparation, incubation, and sampling of salads, it was assumed that few to no Salmonella spp. and S. aureus were present in uninoculated salads during these studies. To confirm this, uninoculated portions of each salad preparation were assayed for the presence of Salmonella spp. and S. aureus at the start and through the duration of each experiment. Ten g of salad were added to 90 ml of 0.1% peptone-water and stomached for 2 min in a Stomacher (Lab-Blender 400, Seward Laboratory, London, England). This preparation was serially diluted in 0.1% peptone-water and surface-plated onto the appropriate medium. Hektoen Enteric agar (Difco) was used to enumerate Salmonella spp. Colonies were green to bluish green after 24 h at 37°C were considered to be salmonellae. Baird-Parker agar (Difco) was used to enumerate S. aureus. Colonies that were black and surrounded by a halo after 48 h at 37°C were considered to be S. aureus. In all instances, uninoculated salads contained <10 Salmonella spp. and S. aureus per 10 g of sample at the start and throughout the course of these experiments.

Salad preparations that were inoculated with S. typhimurium and S. aureus were enumerated on Hektoen Enteric agar and Baird-Parker agar, respectively, using the procedures described previously.

pH determinations

A combination electrode (Beckman H60-10-10) was inserted directly into each salad preparation and pH was measured with a Beckman Model 1500 digital pH meter.

Thermonuclease and enterotoxin determinations

Presence of staphylococcal thermonuclease (TNAse) and enterotoxins was determined for those salads that contained >5 × 10⁵ cells of S. aureus/g. Two procedures were used to determine TNAse activity. In one method (K-D, ref. 6), 20 g of salad were homogenized with 40 ml of water for 2 min in a Waring blender, the pH of the blendure was adjusted to 5.5 with 3 N HCl, and this was heated in boiling water for 20 min. Following heat treatment, the blendure was chilled in ice and centrifuged for 45 min at 10,000 × g. Five microliters of supernatant fluid were added to duplicate 4-mm wells in plates of deoxyribonucleic acid agar (6). The plates were incubated for 1 h at 50°C then flooded with 4 N HCl. Clear zones indicated nuclease activity.

The other method (CAN, ref. 7) involved blending 20 g of salad with 5 g of nonfat dry milk and 40 ml of water, adjusting the pH of the blendure to 3.8 with 3 N HCl, and centurifuging this for 30 min at 23,000 × g. The supernatant fluid was filtered through two layers of tissue paper (Kimwipes®) and 1 ml of 3 M trichloroacetic acid was added to 20 ml of filtrate. This was centrifuged at 23,000 × g for 30 min and the sediment was dissolved in 1 ml of 0.05 M Tris- HCl buffer, pH 9.0. The pH was adjusted to 7.5 with 1 N NaOH and the suspension was heated at 100°C for 15 min. After cooling, 5 μl were dispensed into duplicate 4-mm wells in plates of toluuidine blue DNA agar (8). Plates were incubated for 4 h at 35°C then overnight at room temperature. Wells containing pink zones > 6 mm indicated nuclease activity.

Presence of staphylococcal enterotoxin was determined by ELISA using the procedure of Freed and Bergdoll (M. S. Bergdoll, personal communication).

RESULTS AND DISCUSSION

Bactericidal effect of mayonnaise

Data depicting the fate of S. aureus and S. typhimurium in meat salads prepared with different amounts of mayonnaise and maintained at different temperatures are presented in Tables 1-4. Although S. aureus and S. typhimurium are two very dissimilar bacteria, several similarities were observed in their sensitivities and growth response to different concentrations of mayonnaise in meat salads. Both organisms evidenced a substantial decrease in numbers immediately after they were added to either chicken or ham salad that contained mayonnaise. This is exemplified in Table 1 which illustrates the fate of S. aureus in different preparations of chicken salad. There was a small decrease (0.08 log₁₀) in the number of cells that were inoculated into salad containing no mayonnaise while a 0.3 (50%) and 0.5 (67%) log₁₀ reduction occurred in salads containing one-half and the normal amount of mayonnaise, respectively. A similar response was observed when S. aureus was added to ham salad (Table 2). Those preparations of salad containing mayonnaise evidenced an approximate 1 log₁₀ (88-93%) reduction of cells while a decline of <0.2 log₁₀ (34%) occurred in the salad preparation having no mayonnaise.

Similar results were obtained for S. typhimurium although a greater reduction of cells was observed in the chicken and ham salads that contained no mayonnaise than occurred in similar salads inoculated with S. aureus. Preparations of chicken (Table 3) and ham (Table 4) salads that contained no mayonnaise evidenced a 0.5 (69%) and 0.3 (52%) log₁₀ decrease in cells of S. typhimurium, respectively, immediately after inoculation. However, substantially more cells were inactivated.
when mayonnaise was present. In chicken salad, the population of *S. typhimurium* decreased by 1.0 (90%) and 1.3 (95%) log₁₀ when one-half and the normal amount of mayonnaise were present, respectively, while a 0.4 (64%) and 0.5 (70%) log₁₀ decrease occurred in ham salad prepared with the same respective concentrations of mayonnaise. The data indicate that when mayonnaise is present in a meat salad, upon initial contact, it inactivates a substantial portion of a population of *S. aureus* (30-60%) and *S. typhimurium* (20-25%).

**Effects of storage at 4 C**

Refrigerating the salads at 4 C was an effective means for suppressing the growth of both organisms whether mayonnaise was present or not. None of the preparations of chicken salad inoculated with *S. aureus* (Table 1) or preparations of ham salad inoculated with *S. typhimurium* (Table 4) experienced an increase in numbers of inoculated cells even after 24 h of incubation. Of the salad preparations maintained at 4 C, the greatest increase in cells occurred in a sample of ham salad that was prepared with one-half the normal amount of mayonnaise and inoculated with *S. aureus* (Table 2). A 0.7 log₁₀ increase in cells occurred after 24 h; however, this was still 0.4 log₁₀ cells less than the number that was originally inoculated into the salad.

**Effects of storage at 22 C**

All salads incubated at 22 C, which represented storage at room temperature, exhibited relatively little growth of either *S. aureus* or *S. typhimurium* after 5 h of incubation. The greatest increase of cells occurred in chicken salad that did not contain mayonnaise and was inoculated with *S. aureus* (Table 1). An increase of <0.8 log₁₀ of cells occurred.

When the rates of growth of the two organisms in different preparations of salad after 5 h at 22 C were compared, the data suggested that as the amount of mayonnaise added to the salad was increased, the rate of growth decreased. Results after extending the time of incubation to 24 h adds additional support to this observation. In all instances, substantially less growth occurred at 22 C after 24 h in salads prepared with the actual amount of mayonnaise recommended by their recipes than in equivalent salads that had no mayonnaise. Mayonnaise added to meat salads at one-half the recommended amount retarded growth to an extent that was intermediate to that obtained for salads prepared with no mayonnaise and the recommended amount.

In both types of meat salad, *S. typhimurium* grew better at 22 C than *S. aureus*. For example, in chicken

### TABLE 1. Fate of *S. aureus* in chicken salad containing different amounts of mayonnaise.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Inoculated</th>
<th>Immediately after inoculation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4 C 5 h</th>
<th>24 h</th>
<th>22 C 5 h</th>
<th>24 h</th>
<th>32 C 5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mayonnaise</td>
<td>6.4</td>
<td>3.08</td>
<td>3.00</td>
<td>3.08</td>
<td>3.00</td>
<td>3.75</td>
<td>5.51</td>
<td>3.88</td>
<td>7.04</td>
</tr>
<tr>
<td>1/2 normal portion</td>
<td>6.2</td>
<td>3.08</td>
<td>2.78</td>
<td>2.79</td>
<td>2.63</td>
<td>3.15</td>
<td>5.46</td>
<td>3.43</td>
<td>6.96</td>
</tr>
<tr>
<td>of mayonnaise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal amount</td>
<td>6.1</td>
<td>3.08</td>
<td>2.60</td>
<td>2.58</td>
<td>2.46</td>
<td>2.90</td>
<td>4.30</td>
<td>3.11</td>
<td>6.89</td>
</tr>
</tbody>
</table>

<sup>a</sup>Plated within 10 min after inoculation.

<sup>b</sup>Amount of mayonnaise used was 1/2 of the amount recommended by the recipe.

### TABLE 2. Fate of *S. aureus* in ham salad containing different amounts of mayonnaise.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Inoculated</th>
<th>Immediately after inoculation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4 C 5 h</th>
<th>24 h</th>
<th>22 C 5 h</th>
<th>24 h</th>
<th>32 C 5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mayonnaise</td>
<td>5.6</td>
<td>3.70</td>
<td>3.52</td>
<td>3.11</td>
<td>3.62</td>
<td>2.90</td>
<td>4.32</td>
<td>3.08</td>
<td>6.57</td>
</tr>
<tr>
<td>1/2 normal portion</td>
<td>5.3</td>
<td>3.70</td>
<td>2.56</td>
<td>2.30</td>
<td>3.28</td>
<td>2.78</td>
<td>3.32</td>
<td>2.95</td>
<td>5.80</td>
</tr>
<tr>
<td>of mayonnaise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal amount</td>
<td>5.2</td>
<td>3.70</td>
<td>2.77</td>
<td>2.70</td>
<td>3.04</td>
<td>3.04</td>
<td>3.08</td>
<td>2.95</td>
<td>5.08</td>
</tr>
</tbody>
</table>

<sup>a</sup>Plated within 10 min after inoculation.

<sup>b</sup>Amount of mayonnaise used was 1/2 of the amount recommended by the recipe.
salad prepared without mayonnaise, _S. typhimurium_ evidenced a 3.4 \( \log_{10} \) increase after 24 h while a 2.5 \( \log_{10} \) increase occurred for _S. aureus_. A similar increase was observed when _S. typhimurium_ grew in ham salad prepared without mayonnaise despite the fact that the pH of ham salad was 0.5 units less than that of chicken salad. Unfortunately the pH of the ham salad inoculated with _S. aureus_ was less than that of the ham salad inoculated with _S. typhimurium_, hence growth of the two bacteria in these salads could not be compared directly. The differences in pH of the two different preparations of ham salad were attributed to variation in the acidity of the pickle relish. Although the same style and brand of pickle relish was used to prepare each salad, one lot of relish was more acidic than the other.

**Effects of storage at 32°C**

When salads were held for 5 h at 32°C, which is a temperature that a salad may receive on a hot summer day when enroute to and served at a picnic, neither _S. aureus_ nor _S. typhimurium_ multiplied appreciably. Growth rates for both organisms were very similar to those observed when comparable preparations of salad were held at 22°C for 5 h. However, holding the salads for 24 h at 32°C resulted in the development of larger populations than occurred in equivalent salads that were held at 22°C for 24 h. This is not surprising because 32°C is closer to the optimum temperature for growth of both organisms. As occurred in salads held at 22°C, salads containing mayonnaise and stored at 32°C had substantially lower numbers of _S. aureus_ and _S. typhimurium_ than those prepared without mayonnaise, and salads prepared with an intermediate amount of mayonnaise had intermediate numbers of the two pathogens.

**Effect of mayonnaise on pH of salads**

The antimicrobial properties of mayonnaise have, in part, been attributed to its high acidity for which acetic acid is primarily responsible (10). When mayonnaise is mixed with other food ingredients it reduces the pH of the resulting salad. This is clearly illustrated for chicken salad (Tables 1 and 3) in which the pH of ingredients having no mayonnaise was 6.4 while the pH of the same ingredients mixed with the amount of mayonnaise recommended by the recipe was 6.1. A similar observation was made for the two preparations of ham salad. In one instance the pH of the ham salad containing no mayonnaise was 5.6 while that of the salad prepared with the recommended amount of mayonnaise was 5.2 (Table 2). The other preparation of ham salad had pH values of 5.9 and 5.6 when prepared without and with mayonnaise, respectively (Table 4).

### TABLE 3. Fate of _S. typhimurium_ in chicken salad containing different amounts of mayonnaise.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Inoculated</th>
<th>Immediately after inoculation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>After incubation at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 h</td>
</tr>
<tr>
<td>No mayonnaise</td>
<td>6.4</td>
<td>3.59</td>
<td>3.08</td>
<td>3.20</td>
</tr>
<tr>
<td>1/2 normal portion of mayonnaise&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2</td>
<td>3.59</td>
<td>2.60</td>
<td>2.90</td>
</tr>
<tr>
<td>Normal amount of mayonnaise</td>
<td>6.1</td>
<td>3.59</td>
<td>2.32</td>
<td>2.70</td>
</tr>
</tbody>
</table>

<sup>a</sup>Plated within 10 min after inoculation.

<sup>b</sup>Amount of mayonnaise used was 1/2 of the amount recommended by the recipe.

### TABLE 4. Fate of _S. typhimurium_ in ham salad containing different amounts of mayonnaise.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Inoculated</th>
<th>Immediately after inoculation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>After incubation at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 h</td>
</tr>
<tr>
<td>No mayonnaise</td>
<td>5.9</td>
<td>3.36</td>
<td>3.04</td>
<td>2.95</td>
</tr>
<tr>
<td>1/2 normal portion of mayonnaise&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8</td>
<td>3.36</td>
<td>2.92</td>
<td>2.75</td>
</tr>
<tr>
<td>Normal amount of mayonnaise</td>
<td>5.6</td>
<td>3.36</td>
<td>2.84</td>
<td>2.70</td>
</tr>
</tbody>
</table>

<sup>a</sup>Plated within 10 min after inoculation.

<sup>b</sup>Amount of mayonnaise used was 1/2 of the amount recommended by the recipe.
Both Holtzappfel and Mossel (5) and Gould et al. (3) have reported that decreasing the pH of a meat salad has a negative effect on the ability of \( S. \textit{aureus} \) and \( \textit{Salmonella} \) spp. to survive and grow. Since the presence of mayonnaise reduced the pH of those salads that evidenced suppressed development of \( S. \textit{aureus} \) and \( S. \textit{typhimurium} \), it is likely that the acidity of mayonnaise is an important factor which contributes to its antimicrobial properties in meat salads.

**Production of thermonuclease**

In a previous study, Gould et al. (3) observed that TNase was not detectable in meat salads that contained staphylococcal enterotoxins and populations of \( S. \textit{aureus} \) as large as \( 1 \times 10^5 \) g. Pursuing these observations, salads inoculated with \( S. \textit{aureus} \) were assayed for TNase and enterotoxins. Since approximately \( 10^6 \) cells of \( S. \textit{aureus} \) g of food are required for production of detectable amounts of TNase (1,12) and enterotoxin (12), only those salads that contained more than \( 5 \times 10^3 \) cells of \( S. \textit{aureus} \) g were analyzed for TNase and enterotoxins. For purposes of comparison, two methods were used to determine the presence of TNase, i.e., the method of Koupal and Deibel (6) and the method of the Health Protection Branch of Health and Welfare Canada (7). Results from these analyses are presented in Table 5.

The method of Koupal and Deibel and the Canadian procedure detected TNase in identical samples of meat salads; however, the Canadian procedure appeared to be more sensitive because it produced a stronger response for three of four positive samples. Interestingly, only four of the five samples assayed contained detectable amounts of TNase. The chicken salad that was prepared with one-half of the amount of mayonnaise recommended by the recipe developed a population of \( S. \textit{aureus} \) of \( 9.1 \times 10^5 \) g yet no TNase was detected by either method. These data suggest that the production and/or detection of TNase in meat salads may be erratic; a factor which must be taken into consideration when using the presence of TNase as an indicator of enterotoxin production in meat salads.

**Production of enterotoxin**

Staphylococcal enterotoxin was detected in each of the three preparations of chicken salad assayed (Table 5), including the salad that did not contain detectable amounts of TNase. This suggests that enterotoxin may be present in meat salads in which TNase is not detectable.

Neither of the preparations of ham salad contained detectable amounts of enterotoxin; however, this was not unexpected as only \( 6.3 \times 10^4 \) and \( 3.7 \times 10^4 \) cells of \( S. \textit{aureus} \) were present per g in the two salads. These populations were close to or below the minimum number of cells of \( S. \textit{aureus} \) required to produce detectable amounts of enterotoxins in foods.

**Implications of the results**

Results from these studies clearly illustrate that mayonnaise does not encourage the growth of \( S. \textit{aureus} \) or \( S. \textit{typhimurium} \) in meat salads but rather retards it. This conforms with the observations of Rappaport and Goepfert (9) and Foster (2); however, direct comparisons cannot be made because the salads evaluated by these investigators were of a slightly different composition and had a lower pH than those used for our studies. These results also compare favorably with the observations of Swaminathan et al. (11) who demonstrated that mayonnaise was quite effective in retarding the growth of \( S. \textit{typhimurium} \) when it was spread on the surface of turkey meat and bread to form a sandwich.

In addition to its ability to retard bacterial growth, these studies indicate that mayonnaise in meat salads inactivates a substantial portion of the original population of both \( S. \textit{aureus} \) and \( S. \textit{typhimurium} \). Much of its antibacterial activity may be attributed to its acidity. As previously mentioned, the pH of commercially prepared mayonnaise is quite low, ranging from 3.6 to 4.0. When salads were prepared with the amount of mayonnaise recommended by recipes from a reputable cookbook, a 0.3 to 0.4 unit decrease in pH resulted. Several investigators (3,5) have reported that decreasing the pH of meat salad has an adverse affect on the ability of \( S. \textit{aureus} \) and \( \textit{Salmonella} \) spp. to survive and grow. Hence the acidity of mayonnaise is undoubtedly an important factor that contributes to its antimicrobial properties in meat salads.

Interestingly, relatively little growth (<1 log/g) of either \( S. \textit{aureus} \) or \( S. \textit{typhimurium} \) occurred in any of the meat salads that were held at 22 or 32 C for 5 h. In the case of \( S. \textit{aureus} \), approximately \( 10^6 \) cells/g of food are required for the production of detectable amounts of enterotoxin (14). These data suggest that if staphylococcal food poisoning is to result from the consumption of chicken or ham salads held at temperatures as high as

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**TABLE 5. Thermonuclease and enterotoxin production in salads incubated at 32 C for 24 h and containing greater than 5 x 10^3 cells of S. aureus/g.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( \log_{10} S. \textit{aureus}/g )</th>
<th>K-D</th>
<th>CAN</th>
<th>Enterotoxin^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Salad</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No mayonnaise</td>
<td>7.04</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1/2 normal portion of mayonnaise</td>
<td>6.96</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Normal amount of mayonnaise</td>
<td>6.89</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Ham Salad</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No mayonnaise</td>
<td>6.57</td>
<td>±</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>1/2 normal portion of mayonnaise</td>
<td>5.80</td>
<td>±</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

^a+++, Strongly positive (>10-mm zone diameter); +, moderately positive (7- to 10-mm zone diameter); ±, weakly positive (<7-mm zone diameter); -, negative (no zone).
^b+, Positive; -, negative.
Doyle et al., con't. from p. 156

32 C for less than 5 h, a large population of S. aureus must initially be present or introduced into the salad. Mayonnaise clearly retards the growth of S. aureus and S. typhimurium in meat salads and increasing the concentration of mayonnaise increases the degree of inhibition; however, if meat salads are temperature abused, mayonnaise will not prevent the development of large populations of these organisms or the production of staphylococcal enterotoxin after extended incubation. Hence, addition of mayonnaise should not be considered a substitute for refrigeration for preserving meat salads from the growth of foodborne pathogens.

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Alexander and Marshall. con't. from p. 163

Therefore, the change in water activity due to moisture lost during early incubation did not affect evaporative losses during later incubation. In fact, \( r^2 \) for rate of evaporative loss was .92 or higher for each temperature with Plate Count Agar. Thus, at least 92% of the change in weight at each given temperature was accounted for by time in the incubator.

These results illustrate that composition and quantity of medium, relative humidity in the incubator, position of plates and time of incubation are important variables in evaporative losses during incubation but that the major variable is temperature.

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