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# Relation of the Heat Resistance of Salmonellae to the Water Activity of the Environment[1]

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The effect of water activity ($a_w$) on the heat resistance of eight strains of *Salmonella* was studied. Heat resistance of the organisms increased as the $a_w$ of the heating menstruum was reduced. Sucrose afforded the cells a greater degree of protection than did fructose, glycerol, and sorbitol. A direct correlation between $a_w$ and heat resistance could not be established over the range of $a_w$ levels tested in this study. There was variation among the strains of salmonellae in the magnitude of the increase in heat resistance as the $a_w$ level was reduced. All strains of *Salmonella* tested showed a greater increase in heat resistance than *S. senftenberg* 775W as the environment became drier. Washed cells had *D* values 25 to 75% lower than unwashed cells. Prior growth of the organisms in media with a reduced $a_w$ increased the heat resistance of the organisms when glycerol, but not when sucrose, was the controlling substance.

The high sensitivity of salmonellae to moist heat is widely accepted. It has been documented numerous times that the milk pasteurization treatment is sufficient to kill even exceedingly large numbers of salmonella cells. Recently, Ng and co-workers (5) studied the heat resistance of several serotypes of *Salmonella*. They reported *D* values of 0.9 to 1.3 min at 57 C for most of the strains tested. Two strains, *S. blockley* 2004 and *S. senftenberg* 775W, had *D* values of 5.8 and 31.0 min, respectively.

We recognize a number of factors that influence an organism's resistance to heat. Among these are the *p*H of the medium in which the organisms are heated, the physiological age of the organisms, the composition of the medium in which the cells are grown before heating, and the nature of the heat itself, i.e., moist or dry heat. The fact that moist heat is a more efficient lethal treatment than dry heat is generally appreciated. However, the response of various bacteria to the application of dry heat has not been studied in depth. This facet is taking on more importance with the increasing concern about the presence of certain public health-related microorganisms in food products.

An increase in the heat resistance of bacterial spores as the water activity ($a_w$) of the environment was decreased was reported by Murrell and Scott (4). They found that the heat resistance of spores from six test species was at a maximum at $a_w$ levels between 0.2 and 0.4. The magnitude of the increase in heat resistance of the spores as the $a_w$ was reduced varied greatly among the six organisms.

Relatively little information describing the effect of reduced $a_w$ levels on the heat resistance of nonsporeforming organisms is available. Calhoun and Frazier (1) reported that *Pseudomonas fluorescens* and *Escherichia coli* had an increased resistance to heat when glucose was added to reduce the $a_w$ of the medium in which the cells were heated. Similarly, the heat resistance of *Staphylococcus aureus* was increased when the $a_w$ of the test medium was decreased by the addition of NaCl. The heat resistance of *P. fluorescens* and *E. coli* was also increased by growing the organisms in media having a reduced $a_w$ level before heating them in fresh media at the same $a_w$.

This study was undertaken as a part of an investigation of the behavior of salmonellae in dry and semi-dry environments. One of the principal objectives was to measure the influence of $a_w$ on the heat resistance of salmonellae and to establish or negate a direct correlation between $a_w$ and heat resistance regardless of the chemical composition of the test environment.

## MATERIALS AND METHODS

**Bacterial cultures.** All strains of *Salmonella* and *E. coli* were obtained from the Food Research In-

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stitute culture collection. The culture of *S. alachua* was recently isolated from contaminated nonfat dry milk. Stock cultures were maintained on nutrient agar slants at room temperature. Working cultures were transferred daily in nutrient broth and incubated on a shaker at 32 to 35 C.

**Determination of heat resistance.** A 1.5-ml amount of a 24-hr nutrient broth culture of salmonellae was added to 150 ml of test medium that had been previously equilibrated to 57.2 ± 0.1 C. The heating vessel was a 200-ml, three-necked distillation flask that was immersed in a water bath so that the level of the bath was 1 inch (2.54 cm) above the level of the test medium in the flask. Throughout the heating period, the test medium was agitated by a mechanical stirrer positioned through the center neck of the flask. A glass thermometer was mounted through a second port to monitor the temperature during the test period. The third port was used for introduction of the test organisms and for sampling during the course of the heating period.

**Enumeration of salmonellae.** During the heating period, 1-ml samples were withdrawn and added to 9 ml of diluent at room temperature. Samples (0.1 ml) of appropriate dilutions were surface-plated on duplicate plates of Trypticase soy agar containing 0.2% yeast extract. The plates were incubated at 35 C for 24 hr. A line of best visual fit was drawn through the points plotted on semilog paper. *D* values were obtained from trials in which a minimum of three-log reduction in viable salmonellae occurred.

The diluent was composed of 0.1% peptone water to which sucrose was added to minimize death of salmonellae due to osmotic shock during dilution. Thirty per cent (w/w) sucrose was present in the diluent when solutions having an $a_w$ of 0.96 or greater comprised the test medium. At $a_w$ values of less than 0.96, the diluent contained 45% (w/w) sucrose.

**Preparation of solutions at specific $a_w$ levels.** All test solutions were prepared in 0.01 M phosphate buffer at *p*H 6.9 ± 0.1. The concentrations of sucrose and glycerol necessary to achieve the desired $a_w$ levels were derived from the data of Scott (8). Fructose and sorbitol solutions were prepared by the method of Norrish (6).

## RESULTS

The data summarizing the heat resistance of eight strains of *Salmonella* and a single strain of *E. coli* in sucrose solutions at 57.2 C and *p*H 6.9 ± 0.1 are shown in Table 1. In a separate series of experiments, salmonellae were introduced into a sucrose solution at an $a_w$ of 0.90. This solution was held at room temperature for 3 hr. Viable counts at the end of this period indicated that this brief exposure to reduced $a_w$ levels in itself was not significantly lethal to *Salmonella*.

It is quite obvious that a reduction in the $a_w$ of the environment in which the cells are heated results in an increase in the heat resistance of salmonellae. It is also apparent that the magnitude of the increase in heat resistance varies from strain to strain. From these data, it is possible to place the salmonellae into groups with respect to their heat resistance in sucrose solutions. The "heat-sensitive" group would consist of *S. infantis* and *S. tennessee*. Although both of

TABLE 1. *Heat resistance of Salmonella and Escherichia coli at 57.2 ± 0.1 C and pH 6.9 ± 0.1 in aqueous sucrose*

| Organism | No. of trials | Range of *D* values (min) | Mean *D* value (min) |
|---|---|---|---|
| $a_w$ = 0.99 (15.4% sucrose, w/w) | | | |
| *S. infantis* | 3 | 0.8–1.0 | 0.9 |
| *S. alachua* | 3 | 1.0–1.3 | 1.1 |
| *S. typhimurium* | 4 | 0.7–1.5 | 1.1 |
| *S. anatum* | 4 | 0.8–1.3 | 1.0 |
| *S. anatum* GF | 4 | 1.0–1.2 | 1.1 |
| *S. montevideo* | 3 | 0.7–1.4 | 1.1 |
| *S. senftenberg* 775W | 4 | 13.5–16.0 | 14.5 |
| *S. tennessee* | 3 | 0.6–0.9 | 0.8 |
| *E. coli* | 3 | 0.8–1.5 | 1.2 |
| $a_w$ = 0.96 (39.6% sucrose, w/w) | | | |
| *S. infantis* | 2 | 5–5.5 | 5.3 |
| *S. alachua* | 2 | 20–33 | 26.5 |
| *S. typhimurium* | 6 | 11–19 | 14.3 |
| *S. anatum* | 2 | 33–38 | 35.5 |
| *S. anatum* GF | NT[a] | NT | NT |
| *S. montevideo* | 3 | 18.5–23.0 | 20.0 |
| *S. senftenberg* 775W | 4 | 43–55 | 48.3 |
| *S. tennessee* | 2 | 9–10 | 9.5 |
| *E. coli* | 3 | 8.5–12 | 10.1 |
| $a_w$ = 0.93 (51.3% sucrose, w/w) | | | |
| *S. infantis* | 6 | 12–15 | 14.3 |
| *S. alachua* | 3 | 58–62 | 60.0 |
| *S. typhimurium* | 9 | 15–41 | 30.5 |
| *S. anatum* | 6 | 65–75 | 68.7 |
| *S. anatum* GF | 5 | 40–70 | 56.0 |
| *S. montevideo* | 5 | 50–75 | 58.4 |
| *S. senftenberg* 775W | 3 | 50–60 | 55.0 |
| *S. tennessee* | 4 | 20–25 | 21.3 |
| *E. coli* | 8 | 30–38 | 33.9 |
| $a_w$ = 0.90 (58.6% sucrose, w/w) | | | |
| *S. infantis* | 3 | 17–28 | 21.1 |
| *S. alachua* | 4 | 70–110 | 80.0 |
| *S. typhimurium* | 3 | 40–55 | 46.7 |
| *S. anatum* | 2 | 60–55 | 62.5 |
| *S. anatum* GF | 2 | 53–65 | 59.0 |
| *S. montevideo* | 2 | 65–80 | 72.5 |
| *S. senftenberg* 775W | 5 | 55–75 | 62.0 |
| *S. tennessee* | 2 | 20–27 | 23.5 |
| *E. coli* | 6 | 35–60 | 46.5 |
| $a_w$ = 0.87 (63.7% sucrose, w/w) | | | |
| *S. infantis* | 5 | 19–40 | 26.0 |
| *S. alachua* | 6 | 75–140 | 95.0 |
| *S. typhimurium* | 2 | 60–63 | 61.5 |
| *S. anatum* | 6 | 60–120 | 83.3 |
| *S. anatum* GF | 5 | 75–150 | 94.0 |
| *S. montevideo* | 3 | 65–80 | 75.0 |
| *S. senftenberg* 775W | NT[a] | NT | NT |
| *S. tennessee* | 7 | 25–45 | 35.9 |
| *E. coli* | 4 | 30–70 | 43.7 |

[a] Not tested.

TABLE 2. *Heat resistance of Salmonella and Escherichia coli in aqueous glycerol at 57.2 ± 0.1 C and pH 6.9 ± 0.1*

| Organism | No. of trials | Range of *D* values (min) | Mean *D* value (min) |
|---|---|---|---|
| $a_w$ = 0.99 (4.9% glycerol, w/w) | | | |
| *S. alachua* | 3 | 0.9–1.5 | 1.1 |
| *S. typhimurium* | 2 | 1.1 | 1.1 |
| *S. anatum* | 3 | 0.8–1.1 | 0.8 |
| *S. anatum* GF | 3 | 1.0–1.2 | 1.1 |
| *S. montevideo* | 3 | 1.0–1.2 | 1.1 |
| *S. senftenberg* 775W | 2 | 12.5–14.5 | 13.5 |
| *S. tennessee* | 3 | 0.7–1.3 | 1.1 |
| *E. coli* | 2 | 0.5–1.2 | 0.9 |
| $a_w$ = 0.90 (33.9% glycerol, w/w) | | | |
| *S. alachua* | 3 | 2.1–2.7 | 2.3 |
| *S. typhimurium* | 2 | 1.8–8.3 | 2.6 |
| *S. anatum* | 3 | 2.2–2.5 | 2.3 |
| *S. anatum* GF | 3 | 2.0–4.0 | 2.8 |
| *S. montevideo* | 3 | 2.8–3.1 | 3.0 |
| *S. senftenberg* 775W | 2 | 28.0–35.0 | 31.5 |
| *S. tennessee* | 3 | 1.3–2.3 | 1.7 |
| *E. coli* | 3 | 7.2–8.5 | 8.1 |
| $a_w$ = 0.75 (57.7% glycerol, w/w) | | | |
| *S. alachua* | 3 | 10.5–14.0 | 12.0 |
| *S. typhimurium* | 3 | 8.5–9.5 | 8.8 |
| *S. anatum* | 3 | 5.0–8.5 | 7.0 |
| *S. anatum* GF | 3 | 5.0–6.5 | 5.5 |
| *S. montevideo* | 4 | 6.0–10.0 | 8.0 |
| *S. senftenberg* 775W | 2 | 40.0–43.0 | 41.5 |
| *S. tennessee* | 2 | 5.0–7.0 | 6.0 |
| *E. coli* | 2 | 12.0–14.0 | 13.0 |

these organisms became more resistant as the $a_w$ was reduced, the *D* values obtained at all levels (except at $a_w$ = 0.99 and 0.93) were a factor of 2 less than those characteristic of the other salmonellae. The heat-resistant group would include *S. alachua*, both *S. anatum* cultures, *S. montevideo*, and *S. senftenberg*. Although both *E. coli* and *S. typhimurium* could be included in this latter group, their resistance seems to be intermediary between the two groups.

*S. senftenberg* 775W was significantly more heat-resistant than the other salmonellae when the organisms were heated in phosphate buffer or other media that had an $a_w > 0.99$. However, when the $a_w$ of the environment in which the cells were heated was reduced below 0.99, the magnitude of the increase in heat resistance (as measured by the ratio of the *D* value at the lower $a_w$ to the *D* value at an $a_w$ of 0.99 for that strain) of the other serotypes of *Salmonella* was greater than the increase exhibited by *S. senftenberg* 775W. Similar observations have previously been reported (4). The ratio of the heat resistance of *Bacillus stearothermophilus* to *Clostridium botulinum* type E spores decreased by a factor of $5 \times 10^3$ as the $a_w$ level was reduced < 0.5. This decrease in ratio resulted because of a $10^5$-fold increase in heat resistance of the botulinum spores in contrast to a 20-fold increase in resistance of the *Bacillus* spores as the $a_w$ was lowered. Goepfert and Biggie (3) and Riemann (7) demonstrated that *S. typhimurium* was more resistant than *S. senftenberg* 775W when heated in chocolate and animal feed.

*S. senftenberg* 775W is generally regarded as a laboratory curiosity rather than a significant part of the *Salmonella* problem because of its infrequent isolation from natural products. Because of this, there is some feeling that time-temperature parameters for processing food or feed materials should be based on the heat resistance of other strains of salmonellae. Our data indicate that it is desirable to choose other strains of salmonellae when testing the heat resistance of this genus in dry environments.

It should be pointed out that the response of the individual strains to the increment decreases in $a_w$ is not uniform. For example, *S. anatum* showed the greatest increase in heat resistance when the $a_w$ was reduced from 0.99 to 0.96. Upon further reduction to 0.93, however, the largest increase was manifested by *S. montevideo*. This type of behavior makes it extremely difficult, if not impossible, to be predictive concerning heat resistance changes with regard to $a_w$ changes in a given medium.

One of the major purposes of this study was to establish or negate a direct correlation between the $a_w$ of the environment and the heat resistance of salmonellae. This type of correlation was established with regard to growth of these organisms by Christian and Scott (2), when they demonstrated that the minimum $a_w$ at which salmonellae would grow was independent of the solute used to control the $a_w$.

To ascertain whether the heat resistance of salmonellae was dependent solely on the $a_w$ of the environment rather than its chemical composition, a series of experiments was performed to determine the heat resistance of salmonellae in phosphate buffer to which glycerol was added to establish the desired $a_w$ level. The data obtained in these trials are summarized in Table 2. At an $a_w$ of 0.90, the heat resistance of each serotype of *Salmonella* was only two- to threefold greater than at an $a_w$ of 0.99. In contrast, *E. coli* was seven to eight times more resistant at an $a_w$ of 0.90 than at an $a_w$ of 0.99. Thus, not only was the response of the organisms different from that evidenced in sucrose, but also the pattern exhibited by the individual strains was dissimilar. By comparing the data in Table 2 with those presented in Table 1, it is readily apparent that there is no direct correlation between $a_w$ per se

TABLE 3. *Heat resistance of Salmonella montevideo*[a] *at $a_w$ = 0.96, 57.2 C, pH 6.9 ± 0.1*

| Controlling substance | *D* value[b] (min) |
|---|---|
| Sucrose | 16.5 |
| Glycerol | 1.2 |
| Fructose | 1.3 |
| Sorbitol | 5.5 |

[a] Cells grown at 32 C before heating.
[b] Average of three trials.

and heat resistance of salmonellae. Rather, the heat resistance of salmonellae is dependent on the nature of the substance(s) that is effecting the given $a_w$ level.

The heat resistance of *S. montevideo* was also tested in solutions of fructose and sorbitol at an $a_w$ of 0.96. The *D* values for this organism in sucrose, glycerol, fructose, and sorbitol are presented in Table 3. It is clear that fructose does not afford significant protection to cells and resembles glycerol in this regard. On the other hand, sorbitol is protective but not to the same extent as sucrose. The *D* value of 5.5 min is about one-third of that obtained when *S. montevideo* was heated in a sucrose solution at an $a_w$ of 0.96. This is further evidence that the survival of salmonellae during heating is a function of the composition rather than the $a_w$ of the environment. The implications of these data are clear. That is, the heat resistance of salmonellae in solutions having a reduced $a_w$ (at least within the range of $a_w$ levels covered in this study) cannot be inferred from data collected from tests conducted in a single medium but must be determined experimentally on a controlling substance basis. These observations and conclusions pertain only to solutions at an $a_w > 0.75$. It is entirely possible that, as the $a_w$ is reduced further (e.g., in some dry food products), the controlling substance may be of little or no consequence. The data collected in the present study do not permit any conclusive statements regarding the behavior of salmonellae in those environments. Further, these data show that time-temperature treatments that are to be established for individual substances must be derived from laboratory trials using the substance in question.

Calhoun and Frazier (1) reported that growth of *E. coli* at reduced $a_w$ levels (as controlled by NaCl) afforded the organisms greater protection when heated in solutions in which the $a_w$ was lowered by the addition of sodium chloride. *P. fluorescens* was similarly protected when grown in broth containing glucose to decrease the $a_w$. In contrast, the heat resistance of staphylococci was not affected by prior growth at reduced $a_w$ levels.

*S. montevideo* was grown in nutrient broth containing 10% glycerol ($a_w$ = 0.977). These cells were then heated in phosphate buffer containing glycerol ($a_w$ = 0.96). An average *D* value of 5.6 min was obtained for cells treated in this manner. Comparing this value (*D* = 5.6 min) with that (*D* = 1.2 min) of cells grown in nutrient broth and heated in glycerol at an $a_w$ of 0.96, it is apparent that the heat resistance was increased by prior growth at a reduced $a_w$. When the same experiment was performed substituting sucrose for glycerol in the growth and test medium, somewhat different results were obtained. There was no increase in the heat resistance of the cells grown in the presence of sucrose, and, in fact, the average *D* value (*D* = 11.7 min) was slightly lower than that evidenced by the nutrient broth-grown cells (*D* = 15.9 min). Therefore, it would seem that the mechanism of action of the two carbohydrates is not identical. Work is currently in progress to elucidate further the mechanisms of protection by these and other substances.

These findings are significant in that an investigator who is seeking to determine the heat resistance of salmonellae (or other organisms) in a given environment should be aware that prior growth conditions can markedly influence the results of such determinations. Efforts should be made to select a growth medium that will confer upon the organisms the greatest chance for survival lest the processing schedule derived from the laboratory data prove inadequate for its task.

During the course of this work, we had occasion to wash nutrient broth-grown cells and compare the heat resistance of the washed cells with unwashed cells when both were heated in sucrose solutions. Cells washed three times in cold 0.1% peptone-water became more sensitive to heat. Washed cells had *D* values ranging from 25 to 75% of the *D* values of unwashed cells heated under identical conditions. In addition, washing cells of *S. senftenberg* 775W resulted in reducing or eliminating a lag period (shoulder) that characterized thermal destruction curves of this organism. This observation may in part explain the discrepancies in *D* values obtained by different investigators for salmonellae heated in seemingly similar environments.

## DISCUSSION

The data obtained in this study permit us to draw some important conclusions regarding the heat resistance of salmonellae. First, it is

quite evident that heat resistance is not dependent on the $a_w$ of the environment alone. Rather, the substance(s) that is effecting the reduced $a_w$ is of primary concern. Because of this, it is not possible to determine the heat resistance of salmonellae in one menstruum and apply the data to other media. This conclusion is of considerable importance to the confectionery industry where syrups of different composition are commonplace. Secondly, it appears that the disparity in heat resistance between *S. senftenberg* 775W and other salmonellae disappears as the concentration of certain solutes (e.g., sucrose) is increased. It is quite possible that more serotypes of *Salmonella* will be found that have greater heat resistance than *S. senftenberg* 775W when tested under these conditions.

We can also conclude that the salmonellae are relatively heterogeneous in their response to increased concentrations of sucrose. Among the eight serotypes tested in this study, the establishment of groups on the basis of heat resistance is possible. *S. typhimurium* seemed to be intermediate in *D* value between the two groups, and if additional serotypes are found to possess such intermediate values it may be necessary to add a third group or to eliminate any attempt at such a grouping. It is not possible to draw any broad-based conclusions regarding the relative heat resistance of the genera *Escherichia* and *Salmonella* when only a single culture of the former and eight strains of the latter have been examined. The data do point out that a heat treatment sufficient to destroy *E. coli* may not suffice to inactivate all serotypes of salmonellae. The implications to the food industry of this statement are obvious.

Finally, an insight into the mechanism of protection by various substances may be gained from these observations. Although these experiments are only preliminary in nature, there are indications that more than one mechanism exists. Work is continuing along these lines in an effort to achieve a greater understanding of the effect of heat on microorganisms.

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