Review

Review of Studies on the Thermal Resistance of Salmonellae

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ABSTRACT

Heat resistance data for different serotypes of Salmonella enterica in different food products and laboratory media are reviewed. From all D-values reported, the highest heat resistance of Salmonella was in liquid eggs and liquid egg yolks. The equation from a line drawn through the highest D-values, and above all values reported, was log D-value = 11.7 - 0.183T°C. From this equation, the calculated C-value was 5.3°C (9.5°F), and a process at 71°C (160°F) will require 1.2 s to inactivate 1 log of Salmonella cells. This calculation did not include data that evaluated the heat resistance after stress conditions or data for Salmonella Senftenberg. The heat resistance of Salmonella is highly influenced by the strain tested, the type of experiment (log reduction versus end-point), culture conditions prior to the experiment, heating menstruum, and recovery conditions. Heat resistance data for Salmonella are still nonexistent or scarce in chicken meat, fruit juices, and aquacultured fish.

Various heat treatments utilized by the food-processing industry and the cooking of foods at home are generally effective at destroying vegetative, foodborne pathogenic bacteria. These thermal processes have been developed over time and are based on experimental data on the destruction of bacteria in different foods and in laboratory media. However, occasionally there are salmonellae that survive food-processing techniques. This may result from changes in the formulation or characteristics of foods and alterations in total solids, acidity, or water activity (aw) that affect the thermal tolerance of salmonellae. In addition, some strains of Salmonella are innately more heat resistant, and previous growth and storage conditions can alter the resistance of any particular strain.

The temperature range for growth of salmonellae is between 5.5 and 45°C (41, 111). The maximum temperature for growth, as well as the temperature where salmonellae start to die (thermal processes) depend on the strains of Salmonella present and their growth phase, the food composition and test media, other limiting physical conditions, and the presence of competing microflora. Because of variations in these parameters, it is sometimes difficult to compare data from experiments using different conditions. However, within experiments, it is possible to observe differences in heat resistance among strains and serotypes and the effects of different test conditions. Not all experimental variables have been tested in all relevant foods but some general conclusions can be drawn about how these factors affect heat resistance.

Certain serotypes of Salmonella enterica are notorious for resistance to thermal treatments, the most prominent being Salmonella Senftenberg 775W (111). Although this organism is not an important foodborne pathogen, it is often used as a test organism. The implication is that if a particular thermal process destroys Salmonella Senftenberg 775W, it will also be effective against more common salmonellae in foods. In most experiments with eggs, Salmonella Enteritidis has proven more heat resistant than Salmonella Typhimurium, but this is not always true in culture media (75, 115, 133, 152).

In addition, careful examination of thermal death curves of salmonellae reveals a distinct tailing or biphasic inactivation kinetics under some conditions. This may indicate the presence of two populations of cells, one more heat sensitive than the other (73, 106, 118). The two populations do not appear to be different genetically. Rather, the more resistant cells appear to have manufactured more heat shock proteins (153).

Growth conditions of the test organism also affect their resistance to heat. Cells in the log phase are more susceptible to heat damage than cells in stationary phase (79). Cells grown at higher temperatures or exposed to a sublethal heat shock (25, 133, 153) and those grown at limiting carbohydrate levels (111) are more heat resistant. This increased heat resistance may last for up to 10 h (111). In addition, salmonellae attached to meat surfaces (or to surfaces such as stainless steel or glass) are generally more heat resistant than those that are unattached and dispersed throughout a food or broth. This is relevant for salmonellae attached to food-processing equipment or dishes (43).

Because heating of bacterial cultures may sublethally injure some cells (in addition to killing others), variations
in procedures for recovering and counting heat-treated cells may give rise to different estimates of thermal tolerance. There is generally a lag time from several hours to several days before heat-damaged cells begin measurable growth (111). Incubation of heat-injured cells under anaerobic conditions was found to increase survival (17, 54, 153). Also, incubation at lower temperatures (55) and on rich media rather than on selective media (22, 139) enhanced recovery of injured cells. Between the best and worst media for recovery there may be as much as a 3-log difference in the number of cells recovered.

As to the source of heat, microwave ovens are convenient for heating foods and may be adequate for destroying small numbers of bacteria. However, numerous reports, for various types of foods, document the problems associated with uneven heating by microwave ovens and survival of pathogens. Pung and Cunningham (51), and Heddleston and Doore (64) wrote general reviews on the effects of microwave heating on foodborne pathogens; other experimental results are provided in papers cited in the following sections on milk, poultry, other meats, and other foods.

Heating under reduced or increased pressure and prior irradiation also can enhance thermal destruction of salmonellae. Earnshaw and coworkers (45) reviewed the possibilities for combining heat with other physical inactivation processes, such as ultrasound and pressure, that reduced the heating temperature and time required for killing foodborne pathogens.

The physical and chemical characteristics of foods also affect heat resistance. Increased solids, lower pH (greater acidity), and decreased moisture in foods increase heat resistance (17). Different organic acids (acetic, citric, lactic), used to acidify culture media, were found to have different effects on thermal resistance. A decrease in aw, produced by higher salt or sugar concentrations also enhances resistance (27). As food products, such as milk, eggs, and meats or flours, are dried and moisture levels decline, heat resistance of surviving salmonellae increases dramatically. Ellison et al. (48) developed a mathematical model to describe the thermal inactivation of Salmonella Typhimurium.

Some food additives, including bacteriocins, EDTA, polyphosphates, hydrogen peroxide, and the lactoperoxidase system, make salmonellae more sensitive to heat. Some of these compounds act by production of transitory oxidation products that attack cellular proteins. Others, such as EDTA and polyphosphates, chelate metal ions important for the integrity of cell wall and membranes. Some of these compounds are more effective in culture media than in complex foods, where the compound may interact with fat and protein and be less available to interact with bacterial cells (22, 132).

The presence of high concentrations (>10^6/ml) of other microbes (Escherichia coli, Citrobacter freundii, Pseudomonas fluorescens) in a test medium had a sparing or protective effect on thermal destruction of salmonellae. It was suggested that the high concentrations of nonsalmonellae were interpreted by the cells as a signal to induce transitory-phase gene expression that leads to greater resistance to heat (44). Further investigation of this effect of competitive microflora indicates that protection is probably the result of a rapid decrease in dissolved oxygen (caused by respiration of the added cells) that reduces oxidative damage to the salmonellae (44).

**EXPERIMENTS IN DIFFERENT FOODS**

**Eggs.** Pasteurization of liquid whole eggs, egg whites, and egg yolks has been studied extensively, but less information is available about the conditions necessary for the sterilization of intact shell eggs. The outer shell may harbor many contaminating bacteria from the environment. In addition, Salmonella Enteritidis may be present inside eggs if laying hens are infected with this bacterium. Only a small number of eggs (estimated at about 0.5%) from Salmonella Enteritidis-infected flocks have bacteria within the egg. There are probably <100 to 220 CFU of Salmonella Enteritidis per gram when eggs are first laid. However, these cells can multiply rapidly if the eggs are kept at room temperature. Two main problems are associated with heat-treating shell eggs. High temperatures cause protein coagulation and alter the texture and therefore decrease the possible uses for heat-treated eggs. In addition, transfer of heat to the interior of the egg is relatively slow. This is particularly true when eggs are heated in hot air (55°C); therefore, this process has proved to be impractical. Heating in hot water (57°C) for 25 and 30 min decreases the number of inoculated Salmonella Enteritidis cells but does not completely eliminate them (147). If this treatment is followed by 60 min incubation in hot air, a reduction of 7 log cycles can be achieved (71, 138). Salmonella Enteritidis could be completely inactivated in a water bath at 58°C for 50 to 58 min or by immersion in water at 57°C for 65 to 75 min (131). Changes occurred in the texture of the egg proteins, but the eggs were still useful for some purposes.

The effectiveness of various methods of cooking eggs has been tested by several researchers. Boiling for 15 min to an internal yolk temperature of 88°C and for 7 min to a temperature of 55.4°C effectively killed Salmonella Typhimurium (12, 140). However, soft cooking for 3 to 5.5 min was not sufficient (12, 76, 140). Scrambling of eggs (1 to 1.2 min) and frying over easy (3 to 5 min) were also effective methods of cooking, but eggs fried sunny side up still contained viable salmonellae (12, 76). Salmonella Enteritidis grew rapidly at 23°C, and these cells were more heat resistant than Salmonella Enteritidis from eggs stored in the refrigerator (124). Therefore, storage at room temperature not only allows growth of contaminating bacteria but also increases their heat resistance. When eggs have massive levels of contamination, no cooking method is very effective in destroying salmonellae.

Appropriate conditions for pasteurization of liquid whole eggs, egg whites, and egg yolks have been investigated with respect to a number of variables: pH, aw, and the related variables of salt and sugar concentrations, age and previous growth temperatures of Salmonella cultures used for testing, previous heat shock, storage of eggs at high or low temperatures, concurrent irradiation, and presence of lactic acid, hydrogen peroxide, nisin, EDTA, and polyphosphates. Most data demonstrate that salmonellae survive...
TABLE 1. Thermal resistance of Salmonella in liquid whole eggs

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Condition</th>
<th>D-value (min) at temp (°C)</th>
<th>z-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>No irradiation</td>
<td>33.9a</td>
<td></td>
</tr>
<tr>
<td>Enteritidis</td>
<td>Irradiation (3.9 krad/min)</td>
<td>6.09a</td>
<td></td>
</tr>
<tr>
<td>Enteritidis</td>
<td>Previous culture—broth</td>
<td>9.3a</td>
<td>1.4a</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>Previous culture—broth</td>
<td>6.4a</td>
<td>1.6a</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>Previous culture—egg</td>
<td>16.5a</td>
<td>2.58</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>Heat shock 48°C, 30 min</td>
<td>4.88a</td>
<td></td>
</tr>
<tr>
<td>Typhimurium</td>
<td>No additions</td>
<td>2.3b</td>
<td>2.3b</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Egg + 10% sucrose</td>
<td>4.6b</td>
<td>0.28b</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Egg + yolk + corn syrup</td>
<td>1.6b</td>
<td></td>
</tr>
<tr>
<td>Typhimurium</td>
<td>No additions</td>
<td>34.1b</td>
<td>5.6b</td>
</tr>
</tbody>
</table>

* Schaffner et al. (127). Cells grown to stationary phase in tryptic soy broths (TSB) at 37°C for 72 h.
* Manzini et al. (106). Cells grown to stationary phase in TSB or liquid whole egg, survivors were counted after 3 to 4 days growth on TSA at 35°C.
* Humphery et al. (75). Cells grown to stationary phase in nutrient broth (NB); survivors counted on blood agar plates after 24 h at 37°C.
* Garibaldi et al. (53). Cells grown to stationary phase in triplicate soy yeast extract (TSYE); survivors counted on TSYE agar after 24 to 48 h growth at 35°C.
* Shah et al. (113). Cells grown to stationary phase in TSYE; survivors estimated after growth in TSYE broth at 25°C for 7 days.

...heating better in egg yolks than in egg whites. Thermal resistance varies somewhat among the different serotypes tested (31, 36, 75, 112, 120). Salmonella Sentenben 775W is the most heat resistant of strains tested; Salmonella Enteritidis is more heat tolerant than Salmonella Typhimurium in most studies. Thermal destruction curves for salmonellae in liquid eggs sometimes exhibit significant tailing and heat-injured survivors may recover on appropriate media. Several papers provided data on D-values (decimal reduction times) for thermal destruction of salmonellae in eggs, while other researchers presented data on thermal destruction but did not calculate D-values, or presented them only in a graphical format. Available D-values are presented in Tables 1 through 3.

It should be emphasized that salmonellae present in dried eggs are extremely resistant to the destructive effects of heat, with D-values commonly measured in days rather than in minutes (103, 105).

Brieﬂy, the following conditions tend to increase the heat resistance of salmonellae in eggs: previous heat shock (thermotolerance decayed to normal levels after two to three cell cycles) (99, 133); previous culture in egg rather than broth (109); lower pH (52, 108, 114, 139); incorporation of salt or sugar (30, 31, 52, 115); addition of egg solids (53); low moisture levels (in dried eggs) (103, 105); stationary phase rather than log phase of growth (75).

The following additives or conditions enhanced thermal destruction of salmonellae in eggs: addition of EDTA (52); presence of polyphosphates (52, 90); use of lactic acid instead of HCl to lower pH (52); hydrogen peroxide (108, 114); irradiation (127); cold storage at 4 or 8°C prior to heating (74); addition of the bacteriocin nisin (more effective in culture media than in egg whites) (22).

In addition to the above reports that present experimental data, there are two useful review articles that discuss parameters related to processing of eggs and microbiological contamination (11, 150).

**Milk and dairy products.** Heat resistance of salmonellae in dairy products, particularly milk, has been investigated by a number of researchers. One early paper by Dabbah et al. (35) presented heat survivor curves for eight serotypes of Salmonella Enteritidis. All the curves had extensive tailing, so the authors contended that one should not rely on D-values calculated from the apparently logarithmic part of the heat survivor graphs. Other researchers have calculated D-values, although tailing was observed in other experiments as well.

A greater concentration of total solids in milk increased the heat resistance of Salmonella (42, 46, 91, 99, 104, 105). The Δz-value for Salmonella Typhimurium increased from 4.7 min at 10% solids to 18.3 min at 42% solids (42). The z-values also increased from 4.0°C at 10% solids to 6°C at 42% solids (42). Reduced pressure significantly decreased the heat resistance of Salmonella Typhimurium in milk independently of the milk solid concentration (42). The heat resistance of Salmonella Sentenben did not vary when tested in milk or its 4× retentate (91). Table 4 presents the D-values for destruction of salmonellae in raw milk. Other variables were reported to affect thermal resistance of salmonellae in milk. Experiments performed with cells grown at higher temperatures (43 or 37°C versus 22°C) exhibited a greater heat resistance (42). A heat shock treatment by incubating the cells at 48°C for 30 min prior to the experiment increased their resistance to subsequent thermal treatments (99). Cells heated under reduced air...
TABLE 2. Thermal resistance of Salmonella in liquid egg yolks

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Condition</th>
<th>D-value (min) at temp (°C) of</th>
<th>T-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>64.4</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Yolk only</td>
<td>0.65a</td>
<td>2.22</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Yolk only</td>
<td>0.70b</td>
<td>3.24</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Yolk + 10% NaCl</td>
<td>0.83</td>
<td>4.4</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Yolk + 10% sucrose</td>
<td>0.45</td>
<td>4.6</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>No additions</td>
<td>1.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Enteritidis + Enteritidis</td>
<td>No additions</td>
<td>1.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Sentefenberg, not 775W</td>
<td>No additions</td>
<td>21.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Sentefenberg 775W</td>
<td>No additions</td>
<td>42.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Hampsey et al. (75). Cells grown to stationary phase in NB; survivors counted on blood agar plates after 24 h at 37°C.
* Garibaldi et al. (55). Cells grown to stationary phase in TSYE; survivors counted on TSYE agar after 24 to 48 h growth at 37°C.
* Palumbo et al. (115). Cells grown to stationary phase in tryptose phosphate broth (TPB); survivors determined after 24 h growth on TSA at 37°C.

In some studies, microwaves appeared to eliminate salmonellae in poultry by thermal processes. However, microwave cooking does not reliably destroy salmonellae inoculated onto chickens or turkeys, even though the recommended internal temperature has been reached (2, 15, 94, 125, 129). In some studies, microwaves appeared to eliminate salmonellae in turkey (26), although only one turkey was tested for decontamination after cooking. Salmonella Typhimu-

mum inoculated into chicken loaf, whole roasting chicken, and egg custard was killed by microwaves, but some sal-

monellae survived in microwaved chickenburgers (13).
TABLE 3. Thermal resistance of Salmonella in liquid egg whites

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Condition</th>
<th>D-value (min) at temp (°C) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>51.5</td>
</tr>
<tr>
<td><strong>Enteritidis</strong>a</td>
<td>no nisin; pH 9–9.3</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>1,500 IU nisin/ml</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>2,500 IU nisin/ml</td>
<td>0.55</td>
</tr>
<tr>
<td>Typhimuriumb</td>
<td>pH 7.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>pH 9.2</td>
<td>1.20</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>pH 9.2 + 10% sucrose</td>
<td>1.0</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>No additions</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Enteritidis</strong>c</td>
<td>No additions</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Senftenberg</strong>775Wd</td>
<td>No additions</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Senftenberg</strong>775Wd</td>
<td>pH 9</td>
<td>28.6</td>
</tr>
<tr>
<td><strong>Senftenberg</strong>775Wd</td>
<td>pH 9.5</td>
<td>19.3</td>
</tr>
<tr>
<td><strong>Senftenberg</strong>775Wd</td>
<td>pH 9.5 + PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Derby, Oranieburg,</strong> Typhimuriumf</td>
<td>pH 9</td>
<td>4.38</td>
</tr>
<tr>
<td><strong>Derby, Oranieburg,</strong> Typhimuriumf</td>
<td>pH 9 + PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Derby, Oranieburg,</strong> Typhimuriumf</td>
<td>pH 9.5</td>
<td>3.16</td>
</tr>
<tr>
<td><strong>Derby, Oranieburg,</strong> Typhimuriumf</td>
<td>pH 9.5 + PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium</strong>g</td>
<td>pH 8.2</td>
<td>7.99</td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 9.1</td>
<td>3.17</td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 8.8</td>
<td>2.74</td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 8.8 + H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.87</td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 9.3</td>
<td></td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 8.8</td>
<td></td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 8.2</td>
<td></td>
</tr>
<tr>
<td><strong>Enteritidis, Typhimurium, Senftenberg not</strong>775W</td>
<td>pH 7.8</td>
<td></td>
</tr>
</tbody>
</table>

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<sup>a</sup> Boziaris et al. (22). Cells grown to stationary phase in NB; survivors counted after growth on nutrient agar for 48 h at 37°C.

<sup>b</sup> Garibaldi et al. (52). Cells grown to stationary phase in TSYE; survivors counted on TSYE agar after 24 to 48 h growth at 35°C.

<sup>c</sup> Humphrey et al. (75). Cells grown to stationary phase in NB; survivors counted on blood agar plates after 24 h at 37°C.

<sup>d</sup> Kohl (90). Cells grown in TSYE to stationary phase and then added to pH-adjusted egg whites along with polyphosphate. Survivors were enumerated after growth on TSYE agar. D-values calculated from data in the paper.

<sup>e</sup> Schuman and Sheldon (130). Cells grown to stationary phase in BHI broth and then added to pH-adjusted egg white. Survivors were enumerated after 48 h growth on BHI agar at 37°C.

<sup>f</sup> Palumbo et al. (114). Cells grown to stationary phase in TPB; survivors determined after 24 h growth on TSA at 37°C.
TABLE 4. Thermal resistance of Salmonella in raw milk

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>D-value (min) at temp (°C)</th>
<th>z-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51.8</td>
<td>57.2</td>
</tr>
<tr>
<td>Typhimium, milk**</td>
<td>21.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Typhimium, human**</td>
<td>24.0</td>
<td>0.058</td>
</tr>
<tr>
<td>Human mix**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonhuman mix**</td>
<td>0.068</td>
<td>0.054</td>
</tr>
<tr>
<td>Senftenberg 7775W**</td>
<td>0.122</td>
<td>0.107</td>
</tr>
<tr>
<td>Muenster**</td>
<td>0.097</td>
<td>0.051</td>
</tr>
</tbody>
</table>

**Bradshaw et al. (23). Outbreak-associated strains isolated from milk or humans were grown in TSYE broth to stationary phase, added to raw milk, and heated in small glass tubes. Survivors were counted after 48 h growth on TSYE plates at 37°C.

**D’Aoust et al. (38). Human mix contained Salmonella serotypes: Typhimurium, Infantis, Hadar, Agona, Enteriditis, Heidelberg, Newport, Saint-paul, Thompson, Schwarzengrund. Nonhuman mix included, Salmonella serotypes: Muenster, Kentucky, Anatum, Montevideo, Mbandaka, Altalba, Brandenburg, Senftenberg, Newington, California. Cells were grown in NB at 35°C to stationary phase, inoculated into a bulk tank of raw milk, and a 1-liter sample was heated in a pilot scale pasteurizer. Survivors were pre-enriched in water or milk with brilliant green, enriched in tetrathionate brilliant green and selenite cystine, and numbers were estimated by the most probable number technique.

Beddows (15) reported that pressure cooking of stuffed chicken may not destroy all salmonellae, and the recommended cooking time for stuffed chickens in an electric frypan should be increased.

Commercially cooked turkey rolls heated in water baths at 165°F (73.9°C) for 5.5 h or at 185°F (85°C) for 4.5 h were found to be salmonellae-free. Internal temperatures reached at least 150°F (65.6°C) or were maintained at 145°F (62.8°C) for 4 h (24).

Thermal destruction curves for salmonellae in ground turkey were generated by Cotterill et al. (32) for Salmonella Oranienburg and by Veeramuthu et al. (149) for Salmonella Senftenberg. D-values for Salmonella Senftenberg at 55, 60, and 65°C were 211.4, 13.2, and 3.4 min, respectively. These values were higher than those measured for E. coli O157:H7 under similar conditions and were higher than those reported for some salmonellae in ground beef (Table 5). It is paradoxical that only one report presents D-values of salmonellae in chicken meat. The D-values reported were 0.286 min at 67.5°C and 0.176 min at 70°C (110), which are of the same magnitude as those reported for Salmonella Senftenberg in turkey (149). In fact, of all the serotypes composited for the thermal death time experiments in chicken (Senftenberg, Typhimurium, Heidelberg, Mision, Montevideo, and California), Salmonella Senftenberg was the most heat-resistant (110).

Poultry may become contaminated with salmonellae during slaughter, and numerous procedures have been devised for preventing or removing these bacteria. These slaughterhouse procedures will not be described here. Other decontamination methods (involving heat) targeted at pieces of meat to be sold in grocery stores will be briefly described. A short exposure to steam (20 s) reduced total numbers of aerobic bacteria on the surfaces of whole and cutup chicken and in some cases apparently destroyed all salmonellae. However, many samples were still Salmonella-positive and the chicken had a lightly cooked appearance (39). Other efforts at decontamination used heat in combination with ionizing radiation (144), phosphates (146), alkali (123), and lactoperoxidase (152). All of the chemical treatments combined with elevated temperatures significantly decreased numbers of salmonellae on the chickens, but none effectively inactivated all salmonellae in the meat. Prior γ-irradiation was found to increase killing of salmonellae during subsequent heating.

Other meats. Ground meat is more likely than large pieces of meat to be contaminated with bacterial pathogens and numerous outbreaks of foodborne disease have been traced to ground beef. Salmonellae attached to muscle tissue have a greater heat resistance than those that are attached or that are suspended in broth (81, 146). Other factors increasing the heat resistance of salmonellae in ground meat were prior heat shock (99), added soy proteins (133), and constantly rising temperatures, rather than constant temperatures (145). D-values reported for ground meat heated under various conditions are presented in Table 5.

Frying beef patties for 6 min at 200°C kills bacteria in the surface layer of the patty. However, depending on the level of contamination, it may require 10 min of cooking to destroy salmonellae in the center completely (40). Experiments in cooking ground beef in a microwave demonstrated that all salmonellae could be killed. However, the time and microwave intensity required to cook the meat completely was less than that needed to kill all salmonellae (9).

 Destruction of salmonellae during roasting of beef depends on where bacteria are located, the surface or the interior of the roast, and the heating conditions. Salmonellae injected or inserted into the middle of beef roasts were killed at 54.4°C after 60 min and at 57.2°C after 3 min, when the roasts were processed in hot water (81). However, when salmonellae in the interior of roasts are killed, those on the surface may survive if the meat is dry-roasted (18, 19, 61, 134). Use of steam during part of the dry-roasting process considerably enhanced destruction of salmonellae. It is likely that exposure to steam during part of the dry-roasting process considerably enhanced destruction of salmonellae.
TABLE 5. Thermal resistance of Salmonella in ground beef

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Condition</th>
<th>D-value (min) at temp (°C)</th>
<th>T-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>51.6</td>
</tr>
<tr>
<td>Senftenberg(a)</td>
<td>Untreated</td>
<td>53</td>
<td>15.2</td>
</tr>
<tr>
<td>Thompson(b)</td>
<td>Untreated</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Typhimurium(c)</td>
<td>Heat shocked</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>Typhimurium(d)</td>
<td>Free cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium(e)</td>
<td>Attached to meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium(f)</td>
<td>Constant temp</td>
<td>30.2</td>
<td>2.1–2.7</td>
</tr>
<tr>
<td>Typhimurium(g)</td>
<td>Rising temp 6°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium(h)</td>
<td>Rising temp 8°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium(i)</td>
<td>Rising temp 12.5°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture(j)</td>
<td>No additions</td>
<td>61–62</td>
<td>3.8–4.2</td>
</tr>
<tr>
<td>Mixture(k)</td>
<td>No additions</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Mixture(l)</td>
<td>+ soy protein</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

* Orta-Ramirez et al. (113). Cells were grown to stationary phase in TSB; survivors were enumerated on Petriell coliform plate counts after 24 h of growth at 35°C.
* Mackey and Derrick (99). Cells were grown to stationary phase in TSB. Heat-shocked cells were held at 48°C for 30 min before testing. Survivors were counted after plating on tryptone soy pyruvate agar.
* Humphrey et al. (83). Cells were grown to stationary phase in NB and added to meat just before heating or about 15 min before heating to allow bacteria to attach to meat. Survivors were counted after growth for 24 h at 37°C on blood agar.
* Thompson et al. (145). Cells were grown to stationary phase in BHI and added to meat at either the test temperature or at 24°C and then heated to the test temperature. Survivors were counted after growth for 48 h at 37°C on XLA or XLD agar.
* Goodfellow and Brown (63). Cells were grown to stationary phase in BHI broth; survivors were estimated by most probable number techniques or after growth for 48 h at 37°C on plate counting agar (PCA) with an overlay of XA agar. Mixture included Salmonella serotypes: Agona, Bovis-morbificans, Muenchen, Newport, Typhimurium.
* Craven and Blankenship (33). Cells were grown to stationary phase in BHI. Survivors were enumerated after growth on PCA with an overlay of XA agar for 48 h at 37°C. Mixture included Salmonella serotypes: Brandenburg, Lexington, Manhattan, Muenchen, Newport.

Salmonellae on the surface of the roasts and allowed safe production of rare roast beef (30, 61).

Microwave cooking of ham and pork slices and chicken drumsticks, inoculated on the surface with Salmonella senftenberg, was an effective method for killing these bacteria. Results for chicken were better when the meat was wrapped in polyvinylidene chloride, perhaps because this wrap retained steam near the surface of the meat (125). Attempts to enhance thermal destruction of salmonellae on the surface of pieces of beef by dipping the meat into solutions of 3% acetic or lactic acid at 70°C for 15 s met with some success. Acetic acid appeared to be more effective than lactic acid, but neither treatment resulted in greater than a 2-log decrease in numbers of Salmonella Typhimurium (3, 4).

Survival of salmonellae in meat processed into jerky and various sausages depends on a number of factors: fermentation that produces an acidic environment, drying, concentration of salts and inhibitors, and heating. Examination of the processes for making Lebanon bologna (47), Thuringer sausage (57), and pepperoni (135) revealed that fermentation alone (to a pH of 4.5 to 4.7) was not enough to kill all contaminating salmonellae; a heating step was required to achieve a >7-log kill. Production of a nonfermented snack sausage also required a heating step of at least 53.9°C for 3.5 h to kill salmonellae (136). The thermal processing steps normally employed in processes for making summer sausage (102), frankfurters (116), and beef jerky (63, 70) effectively destroy salmonellae.

Frying of bratwurst at 180°C for 4 min or at 130°C for 6 min eliminates contaminating salmonellae (128). Convritional grilling and roasting methods for cooking a Turkish meat, korkaç (lamb intestine), were also effective in killing salmonellae, although the potential for postcooking contamination of this meat sold at roadside stands was significant (59).

Irradiation at 0.8 kGy prior to reheating of minced cook–chill roast beef and gravy was somewhat effective at decreasing D-values for Salmonella Typhimurium (62). Radiation apparently rendered the surviving cells more heat sensitive. However, this increased heat sensitivity declined after the irradiated meat was kept in refrigerated storage (2 to 3°C) for 14 days. Therefore, it appears that preirradiation would not significantly increase safety (as regards contamination with Salmonella) of minced cook–chill roast beef. Table 6 presents the D-values obtained in these experiments.

Chocolate. Although chocolate may seem an unlikely food vector for salmonellosis because of its low moisture and high sugar content, several outbreaks traced to chocolate products demonstrated that these characteristics and the usual heat treatment during processing may be insufficient for the elimination of all salmonellae. Under low moisture conditions, heat resistance of bacterial contaminants is greatly enhanced as seen in experiments with melted chocolate. Heat resistance of Salmonella Typhimurium was found to be even greater than that of Salmonella Senften-
berg 775W when tested in molten milk chocolate (56). Even at 90°C, it took more than 1 h to kill 90% of Salmonella Typhimurium and at least 50 min for similar destruction of Salmonella Senftenberg. The z-values reported were similar for both organisms, 19°C for Salmonella Typhimurium and 18°C for Salmonella Senftenberg. Addition of moisture to melted chocolate in the form of a distilled water spray or mist dramatically decreased D-values of Salmonella Anatum measured at 71°C (14). The D-value was 20 h with no added moisture, and it decreased to 4 h with 2% added moisture. However, when high levels of contaminating bacteria were added, the calculated time for elimination was insufficient, perhaps because water was evaporating from the chocolate during the prolonged heating. D-values for different Salmonella serotypes in chocolate are presented in Table 7. D-values for Salmonella Typhimurium in samples of chocolate syrup with water activities of 0.75 to 0.84 varied from 1.2 to 3.2 min at pH 5.1 and 5.35, respectively, and the z-values varied from 0.2 to 7.7°C (141). Data on thermal resistance of salmonellae with reference to chocolate processing were further discussed in a review by D’Aoust (37).

Wheat flour, corn flour, and corn-soy milk blends. As noted earlier, in discussions on powdered milk and spray-dried eggs, salmonellae in dry foods are much more resistant to heat than those in moist environments. Thermal destruction curves for Salmonella Weltevreden in wheat flour at different aw showed a rapid death rate in the first 5 to 10 min followed by a slower, linear decrease (8). Irrespective of the initial aw (from 0.2 to 0.6), heating at 57 to 75°C caused evaporation of most of the water to an aw of 0.2 within 5 to 10 min (period of high death rate). D-values calculated from the period of slower population decline demonstrated that salmonellae in flour at an aw of 0.3 to 0.4 had the maximum heat resistance. At an aw of 0.4, the measured D-value was 875 min at 60 to 62°C and while at an aw of 0.5 the D-value at this temperature was about 100 min. Therefore, the lower the initial moisture level, the longer it will take to kill all the salmonellae present.

A similar pattern of results was obtained in experiments with corn flour. Destruction of eight Salmonella serotypes inoculated onto corn flour (10 to 15% moisture) was compared at ambient temperature (25°C) and at an elevated storage temperature (49°C). At 49°C, D-values ranged from 0.3 h (Salmonella Newington) to 9.9 h (Salmonella Tennessee). At ambient temperatures, the measured D-values were 5- to 10-fold longer. D-values were also greater at lower moisture levels (148).

Corn-soy milk blends, developed as supplements for infants and children in areas with inadequate protein in the diet, were heat treated at various temperatures to determine an optimum procedure for destroying salmonellae without significantly affecting the vitamins and other nutritional components (20, 21). Heating at 43 or 49°C for 13 and 10 days, respectively, was found to eliminate Salmonella Anatum and Salmonella Senftenberg added to the blends. Con-
taminating cells were destroyed more rapidly at higher tempera-
tures, but nutritional losses were excessive. Heating this
protein blend to 61.4 to 67.2°C with a continuous micro-
wave tunnel also effectively killed Salmonella Senftenberg.
If the blend was cooled to ambient temperatures within 24 h,
nutritional losses were minimal.

Shellfish. There are only two reports that investigated the
heat resistance of salmonellae in shellfish. The first dis-
cussed survival of heat-resistant Salmonella Senftenberg 775W in
oysters (60). D-values for Salmonella Senftenberg in
homogenized oysters ranged from 35 to 0.3 min at 56 and
70°C, respectively. Cells were more heat resistant if
added to the cold homogenate and heated than if they were
added to a preheated homogenate. D-values for oysters in
the shell were slightly higher than for homogenates.

Andrews and Wilson (5) examined the effects of cook-
ing on several serotypes in the Moroccan food snail (im-
ported to the United States and used as escargot). Salmo-
nellae in the snails were killed by bringing the snails slowly
to a boil and then simmering for 6 min, the recommended
cooking procedure. Internal temperature reached 200°F
(93°C). In both shellfish experiments, the temperatures of
the animals inside the shells were nearly the same as the
temperatures of the cooking water. Therefore, the shell did
not appear to be a significant barrier to heat transfer.

Coconut. Pasteurization of raw coconut meat can be
accomplished by incubation in a water bath at 80°C for 8 to
10 min (126). This method yields a product acceptable
for commercial use.

Other foods. Some other foods that cannot be cate-
gorized easily will be discussed here. These include con-
venience foods heated in microwaves and mixed dishes
such as stews and soups heated in slow cookers. Green bean
casserole, baked navy beans, chicken cacciatore, barbecued
ribs, and pork pot roast were all found to be salmonella-
free when cooked in slow cookers for the recommended
times (122).

Microwave cooking of convenience foods was not al-
ways adequate to destroy bacterial contaminants. Various
factors were found to affect sterilization of foods. Micro-
wave heating of single servings of tomato soup for a given
time resulted in the highest temperatures in the middle of
the soup, lowest at the top, and intermediate at the bottom
of the container. Nevertheless, Salmonella Typhimurium
was killed more rapidly in the top than in the lower ranges
of the soup (34).

Microwaving of mashed potatoes inoculated with Sal-
monella Cabana killed the bacteria if cooking time was
long enough. But at the recommended cooking time, some
cells remained viable. Addition of whole peas to the pota-
toes significantly reduced microbial killing by allowing for-
mation of micropockets with lower temperatures (117).

Other experiences in reheating mashed potatoes, beef stro-
ganoff, and baby foods demonstrated that microwave ovens
do not reliably kill Salmonella Typhimurium (142).

Tests with three microwave models and 30 ready-pre-
pared foods belonging to the cook-chill, sterilized, and
cook-freeze groups measured survival of added Salmonella
Typhimurium. Viable cells were recovered after cooking for
at least the recommended time or intensity from all samples
except special fried rice and Christmas pudding (41).

Characteristics of food related to efficiency of micro-
waves for killing of Salmonella were investigated by Hed-
dixon et al. (66–69). Using microwave heating times suf-
ficient to induce a final mean mean temperature of 60°C,
destruction of salmonellae ranged from 3.17 (in ultrahigh
temperature milk) to 0.44 log CFU/ml (in beef broth). High
sodium content of foods appeared to be the primary factor
causing nonuniform heating temperatures in foods that re-
sulted in increased survival.

EXPERIMENTS USING CULTURE MEDIA

In addition to the above research describing heat resist-
tance of Salmonella in real foods, other researchers exam-
ined heat resistance of various serotypes in defined culture
media under various conditions. Tables 8 through 13 pre-
sent almost all of the available data on D-values measured in
culture media. In brief, the experiments investigated effects of
the following parameters:

Different culture media. Tables 8 and 9 provide D-
values for salmonellae tested in different media. In most
cases, a rich growth medium was used. It is difficult to
compare data from different experiments because of differ-
ences in the serotypes of Salmonella used, in protocols for
testing, in growth media, and in procedures for enumerating
survivors. However, within experiments, it is possible to

Alfalfa seeds. Outbreaks of salmonellosis have been
 traced to contaminated sprouts, so some attention has been
focused on methods for sterilizing seeds or sprouts. Ther-
mal treatments of seeds, of course, have the potential for
destroying the viability of the seeds. Indeed, experiments by
LaRoue et al. (82) revealed that incubation of seeds at
57 to 60°C for 5 min could destroy all inoculated Salmo-
nella Stanley, but even slightly higher temperatures or lon-
ger exposures to these temperatures significantly decreased
germination of the seeds. Because the timing and tempera-
ure range are so narrow, this procedure may not be useful
commercially.
<table>
<thead>
<tr>
<th>Medium</th>
<th>D-value (min) at temp (°C) of:</th>
<th>z-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52</td>
<td>54.4</td>
</tr>
<tr>
<td>CASO-YE</td>
<td>5.3</td>
<td>2.7</td>
</tr>
<tr>
<td>LEMCO</td>
<td>1.9–3.1 (lr)</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>18.9–32.5 (ls)</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>4.95</td>
<td>6.3</td>
</tr>
<tr>
<td>MM</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>NB</td>
<td>6.28 (lr), 11.76 (dl)</td>
<td>3.62 (lr), 9.14 (dl)</td>
</tr>
<tr>
<td>TSBYE</td>
<td>9.98</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* CASO-YE, casein peptone–soymeal peptone broth with yeast extract; LEMCO, Lemco broth; LB, Luria–Bertani medium; MM, minimal media; TSBYE, tryptic soy broth with yeast extract; (l), log phase; (s), stationary phase; (lr), linear; (tl), tail.

1. Xavier and Ingham (153). Cells were grown to stationary phase; survivors were counted after 48 h growth on nutrient agar at 25°C.
2. Humpheson et al. (73). Cells grown to stationary phase; survivors were counted after 48 h growth on NB agar at 37°C. Thermal death curves were biphasic so D-values were given for periods of rapid linear decline and slower tail decrease.
3. Shab et al. (133). Cells grown to stationary phase; survivors were counted after 7 days growth on TSBYE agar at 25°C.
4. Humphrey et al. (79). Cells were grown to log or stationary phase; survivors were counted on blood agar plates after growth at 37°C for 24 h.
5. Dhir and Dodd (47). Cells grown to stationary phase; survivors were counted after 48 h growth on LB agar at 37°C.
6. Humphrey et al. (78), as in footnote e.
7. Humphrey et al. (77), as in footnote e.
8. Bozans et al. (22). Cells grown to stationary phase; survivors were counted after 48 h growth on NB agar at 37°C.
TABLE 9. Thermal resistance of Salmonella in different culture media

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Medium</th>
<th>50</th>
<th>52</th>
<th>54.4</th>
<th>55</th>
<th>57</th>
<th>59.2</th>
<th>58</th>
<th>60</th>
<th>62</th>
<th>65</th>
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<tr>
<td>Typhimurium*</td>
<td>TSIB</td>
<td>&gt;60.0</td>
<td>20.0</td>
<td>14.0</td>
<td>5.0</td>
<td>0.3</td>
<td>7.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Typhimurium*</td>
<td>BHI</td>
<td>18.3</td>
<td>4.6</td>
<td>0.82</td>
<td>0.4</td>
<td>4.3</td>
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</tr>
<tr>
<td>Typhimurium*</td>
<td>PO2</td>
<td>3.7</td>
<td>0.06</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Senftenberg*</td>
<td>PO2</td>
<td>14.23</td>
<td>6.23</td>
<td>2.69</td>
<td>7.7</td>
<td></td>
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<td></td>
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<tr>
<td>Senftenberg*</td>
<td>PO3</td>
<td>17.13</td>
<td>7.14</td>
<td>2.88</td>
<td>7.2</td>
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<td></td>
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<tr>
<td>Senftenberg*</td>
<td>PO4</td>
<td>19.32</td>
<td>3.72</td>
<td>3.06</td>
<td>6.9</td>
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<tr>
<td>Senftenberg*</td>
<td>PO5</td>
<td>12.77</td>
<td>5.39</td>
<td>2.31</td>
<td>7.5</td>
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<tr>
<td>Senftenberg*</td>
<td>PO6</td>
<td>15.14</td>
<td>5.86</td>
<td>1.92</td>
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<td></td>
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<tr>
<td>Senftenberg/</td>
<td>HI</td>
<td>268</td>
<td>36.2</td>
<td>6.3</td>
<td>6.8</td>
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<tr>
<td>Senftenberg/</td>
<td>HI</td>
<td>146</td>
<td>4.9</td>
<td>0.62</td>
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<tr>
<td>Bedford*</td>
<td>HI</td>
<td>350</td>
<td>18.8</td>
<td>4.3</td>
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<tr>
<td>Bedford*</td>
<td>HI</td>
<td>88</td>
<td>5.5</td>
<td>0.49</td>
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<tr>
<td>Agama*</td>
<td>BHI</td>
<td>0.96</td>
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<tr>
<td>Dohy*</td>
<td>BHI</td>
<td>0.66</td>
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<tr>
<td>Infantis*</td>
<td>BHI</td>
<td>0.75</td>
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<tr>
<td>London*</td>
<td>BHI</td>
<td>0.56</td>
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</tbody>
</table>

* Wolfson et al. (152). Cells grown to stationary phase; survivors recovered on PCA with pyruvate after 48 h at 37°C.
* Davidson and Willy (40). Cells grown to stationary phase; survivors recovered on tryptose blood agar with oxalated horse blood after 48 h at 37°C.
* Corry and Roberts (29). Cells grown to stationary phase; survivors recovered on PCA with pyruvate after 48 h at 37°C.
* Corry (28). Cells grown to stationary phase; survivors recovered on PCA with pyruvate after 3 days at 37°C.
* Liu et al. (93). Cells grown to stationary phase; survivors recovered on TSA after 48 h at 37°C.
* Baird-Parker et al. (10). Cells grown to stationary phase; survivors recovered on peptone–Lenco agar with oxalated horse blood after 48 h at 37°C.

Observe variation in heat resistance between different strains and serotypes.

Recovery procedures. Heating of bacterial cultures kills some of the population and may sublethally injure other cells. Depending on how these cells are treated after the heat challenge, they may survive to be counted or they may die. Incubation of heat-injured cells under anaerobic conditions increased survival (17, 54, 153). Also, incubation at lower temperatures (34) and on nonselective, rich media rather than on selective media (22) enhanced recovery of injured cells.

High sugar concentrations. These effectively lower aw, partially dehydrate cells, and increase heat resistance (10, 27, 28, 55, 58, 72, 141). The z-values reported at aw of 0.85 and 0.98 were 19.0°C and 10.7°C, respectively (54). For an aw of 0.9, z-values were reported as 16.5°C (35) or 8.9°C (10). Table 10 presents data on heat resistance of Salmonella Typhimurium in sucrose solutions with aw levels of 0.83 to 0.99. Differences in the D-values reported were attributed to differences in the strains used (141) and yielded very different z-values. Table 11 includes similar but less extensive data for other serotypes. Reducing the aw of phosphate buffer (pH 6.9) to 0.75 using glycerol also decreased the heat resistance of Salmonella Typhimurium (58). The effect of glucose, glycerol, glucose/sucrose, and glycerol/sucrose solutions (aw 0.85 to 0.95) on the heat resistance of Salmonella was also studied by Corry (28). Reducing the aw with glycerol alone was more effective in reducing the heat resistance of Salmonella Typhimurium than glucose alone or different combinations of glycerol/ sucrose, or glucose/sucrose (28). Effects of some other sugars (fructose and sorbitol) were also measured by Goepfert et al. (58) and El-Gazzar and Marth (46).

High salt concentrations. These have effects similar to sugar (10, 17, 50, 67, 69, 107, 112).

Drying. This is also a case of decreased aw. As a product is dried and moisture levels decline, heat resistance can dramatically increase (6, 7, 27, 72, 88, 121).

Sublethal heat shock. This increases heat resistance by 2.6- to 20-fold for up to 10 h (25, 100, 153). Table 12 compares D-values for untreated and heat-shocked Salmonella Typhimurium and Salmonella Enteritidis.

Other temperature effects. Prior storage at higher temperatures or exposure to slowly increasing temperatures during testing increased the heat resistance of salmonellae (29, 74, 78, 98).

pH. Higher pH conditions (alkaline) enhance thermal destruction of salmonellae while more acidic conditions enhance resistance. In addition, different organic acids used to acidify culture media had a greater or lesser effect on thermal resistance (17, 77, 80, 87, 93, 143). The heat resistance of Salmonella Enteritidis was lower when acetic acid was used than hydrochloric, than lactic, than citric acid when used at the same concentration and pH (17). D-values...
of Salmonella Enteritidis grown to log phase in nutrient broth and then incubated for 30 min at different pH values before the thermal death time experiment showed that the heat resistance increases from pH 7 (D_{50°C} = 11.4 min) to its highest at pH 9.5 (D_{50°C} = 25.0 min), and then decreased at higher pHs (pH 10.5, D_{50°C} = 11.4 min) (80).

Lactoperoxidase system. This naturally occurring system includes lactoperoxidase, potassium thiocyanate, and hydrogen peroxide that interact to produce several transitory oxidation products that attack cellular proteins. Exposure to lactoperoxidase decreased D-values significantly but increased the z-value from 7.3°C (heat only) to 9.5°C (heat with lactoperoxidase) (Table 13). This effect is more pronounced at lower cell concentrations (151).

**Competitive microflora.** Further investigation of the effect of competitive microflora demonstrated that the stationary-phase adaptive response was induced too slowly to account for the rapid increase in thermotolerance when competitors were added to Salmonella Typhimurium cultures. Instead, it appears that a rapid decrease in dissolved oxygen (caused by respiration of the added cells) may reduce oxidative damage to the salmonellae, allowing greater survival (1, 44) (Table 13).

**Surface attachment.** Salmonella Enteritidis attached to glass and stainless steel exhibited a twofold increase in D-values at 52°C, as compared to unattached cells. This is relevant for salmonellae attached to food-processing equipment or dishes and may be related to the increased

---

**TABLE 10. Thermal resistance of Salmonella Typhimurium in sucrose solutions**

<table>
<thead>
<tr>
<th>Water activity (aw)</th>
<th>D-value (min) at 52°C</th>
<th>60°C</th>
<th>65°C</th>
<th>z-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.99</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.98</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.96</td>
<td>14.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.95</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.94</td>
<td>4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.93</td>
<td>30.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.90</td>
<td>46.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.89, 0.892</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.87</td>
<td>61.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.85, 0.853</td>
<td>31.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.83</td>
<td>40.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Goepfert et al. (58). Cells grown to stationary phase; test medium was sucrose in phosphate buffer; survivors recovered on TSYE agar after 24 h at 35°C.
* Gibson (55). Cells grown to stationary phase; test medium was sucrose in deionized water; survivors recovered on blood agar after 6 to 8 days at 30°C.
* Corry (28). Cells grown to stationary phase; test medium was sucrose in phosphate buffer; survivors recovered on PCA with pyruvate after 5 days at 37°C.
* Sumner et al. (141). Cells grown to stationary phase; test medium was sucrose in phosphate buffer; survivors recovered in lactose broth at 30°C for 48 h and then plated on Hektoen enteric agar.

**TABLE 11. Thermal resistance of Salmonella in sucrose solutions**

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>D-value (min) at 52°C</th>
<th>60°C</th>
<th>65°C</th>
<th>z-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaligenes</td>
<td>1.1</td>
<td>80.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatum</td>
<td>1.0</td>
<td>62.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedford</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infantis</td>
<td>0.9</td>
<td>21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montevideo</td>
<td>1.1</td>
<td>72.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunnysberg</td>
<td>14.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennessee</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Goepfert et al. (58). Cells grown to stationary phase; test medium was sucrose in phosphate buffer; survivors recovered on TSYE agar after 24 h at 35°C.
* Baird-Parker et al. (10). Cells grown to stationary phase; test medium was sucrose in HI broth; survivors recovered on peptone-Lemco agar with oxidase horse blood after 48 h at 37°C.
* Gibson (55). Cells grown to stationary phase; test medium was sucrose in deionized water; survivors recovered on blood agar after 6 to 8 days at 30°C.
**TABLE 12. Effects of different heat treatments on the thermal resistance of Salmonella**

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Condition or additive</th>
<th>D-value (min) at temp (°C) of:</th>
<th>52</th>
<th>54</th>
<th>56</th>
<th>57.2</th>
<th>57.6</th>
<th>58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium*</td>
<td>No heat shock</td>
<td></td>
<td>21.3</td>
<td></td>
<td></td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium*</td>
<td>Heat shock 30 min at 42°C</td>
<td></td>
<td>96.1</td>
<td></td>
<td></td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium*</td>
<td>Heat shock 30 min at 52°C</td>
<td></td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>No heat shock, aerobic</td>
<td></td>
<td>5.3*</td>
<td>2.7*</td>
<td>1.3*</td>
<td>1.8*</td>
<td>0.9*</td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Heat shock 60 min at 42°C, aerobic</td>
<td></td>
<td>16.9</td>
<td>4.5</td>
<td>2.5</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Heat shock 60 min at 42°C, anaerobic</td>
<td></td>
<td>20.0</td>
<td>6.1</td>
<td>3.2</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Heat shock 30 min at 48°C</td>
<td></td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Cells grown at 20°C</td>
<td></td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Cells grown at 37°C</td>
<td></td>
<td>2.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Cells grown at 44°C</td>
<td></td>
<td>14.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Bunning et al. (25). Cells were grown in TSYE broth; survivors were counted on TSYE agar plates after growth at 25°C for 7 days.
* Xavier and Ingham (153). Cells were grown to stationary phase; survivors were counted after 48 h growth on nutrient agar at 25°C under aerobic or anaerobic conditions.
* Shah et al. (153). Cells grown to stationary phase in TSYE; survivors estimated after growth in TSYE broth at 25°C for 7 days.
* Humphrey et al. (78). Cells were grown to log phase; survivors were counted on blood agar plates after growth at 37°C for 24 h.

Heat resistance observed for salmonellae attached to meat (43, 79). See Table 13 and the section on meat.

Chelating agents. Polyphosphates chelate metal ions important for the integrity of cell walls and membranes. Their presence in a test medium enhances sensitivity of cells to heat (122).

Other food additives. Some amino acids and sugars increase heat resistance, while other compounds, such as cysteine, glutathione, sodium citrate, and glucose decrease heat resistance (106, 107). Addition of the bacteriocin nisin with egg proteins and is less available to interact with bacterial cells (22) (see also Tables 3 and 13). Pediocin has also been used in conjunction with heat and pressure to inactivate foodborne bacteria (85).

Other conditions. Cells grown in culture media with low levels of carbohydrates are more heat resistant (111). Irradiation and heat apparently injure cells by different mechanisms. Exposure to one appears to lower the resistance to the other condition (86). Increased hydrostatic pressure caused death of salmonellae, and, in combination with heat, high pressure elevated the amount of thermal destruction of foodborne bacteria (85).

**TABLE 13. Effect of other condition/additives on the thermal resistance of Salmonella**

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Condition or additive</th>
<th>D-value (min) at temp (°C) of:</th>
<th>50</th>
<th>52</th>
<th>55</th>
<th>58</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium*</td>
<td>Heat only</td>
<td></td>
<td>20</td>
<td>14</td>
<td>5.0</td>
<td>0.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Typhimurium*</td>
<td>Heat with lactoperoxidase</td>
<td></td>
<td>12</td>
<td>7.5</td>
<td>4.0</td>
<td>2.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Typhimurium*</td>
<td>Competitive flora &lt;10/mld</td>
<td></td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium*</td>
<td>Competitive flora &gt;10/mld</td>
<td></td>
<td>2.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>No nisin</td>
<td></td>
<td>6.3 (2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>With 500 IU nisin/ml</td>
<td></td>
<td>5.4 (2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>With 1000 IU nisin/ml</td>
<td></td>
<td>5.0 (2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>With 1500 IU nisin/ml</td>
<td></td>
<td>4.3 (2.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Unattached, planktonic cells—LB</td>
<td></td>
<td>4.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Attached to glass—LB</td>
<td></td>
<td>10.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Attached to stainless steel—LB</td>
<td></td>
<td>9.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Unattached, planktonic cells—MM</td>
<td></td>
<td>3.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Attached to glass—MM</td>
<td></td>
<td>9.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritidis*</td>
<td>Attached to stainless steel—MM</td>
<td></td>
<td>8.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Wolfson and Sarnes (155). Cells grown to stationary phase; survivors recovered on PCA with pyruvate after 48 h at 37°C.
* Duffy et al. (44). Cells grown to log phase in LB; survivors counted on TSA.
* Bozian et al. (22). Cells grown to stationary phase; survivors were counted after 48 h growth on NB agar at 37°C. D-values between parentheses were measured from growth on XLD agar.
* Dhir and Dodd (43). Cells grown to stationary phase and exposed to glass or stainless steel in LB or MM; survivors were counted after 48 h growth on LB agar at 37°C.
CONCLUSIONS

Salmonellae are certainly sensitive to heat, but that sensitivity can vary greatly. In particular, it should be noted that salmonellae in dried food products are extremely heat resistant and may act more like spores in their thermal tolerance. In addition, salmonellae in foods left at room temperature for a time are more heat resistant than those that have been surviving in a cold refrigerator. This is one more reason to be cautious about even moderate temperature abuse of a food or food ingredient. Acidity or alkalinity and concentrations of sugars and salt in foods will also significantly affect thermal tolerance and should therefore be monitored during food production. Finally there are some foods that are difficult to sterilize by heat treatment, such as packaged vegetables that are to be used fresh in salads, nuts, and shell eggs. Excessive heat alters the texture or flavor of these foods. For these foods, as well as for dried foods, treatment with irradiation or addition of additives or some other preservatives may sufficiently weaken or kill some salmonellae so that a lower temperature can be used to finish the processing and make the food safe.

One promising approach involves the use of combinations of heat with other preservation methods such as bacteriocins, chelating agents, irradiation, or pressure treatments. A plot of all D-values (log) reported versus temperature without including data for Salmonella Senftenberg, nor data for heat resistance after stress conditions or added salts or solids (not shown), indicate that the highest heat resistance for salmonellae was in liquid eggs and liquid egg yolks. The calculated equation from a line drawn above all values was log D-value = 11.7 – 0.188T°C. From this equation, the calculated z-value was 5.3°C (95%), and a process at 71°C (160°F; milk pasteurization temperature) will require 1.2 s to inactivate 1 log of Salmonella cells. The calculated condition for inactivation is three times lower than that calculated from a fitted line of data collected on Listeria monocytogenes (97). The process required to inactivate Salmonella in specific products will vary depending on the food substrate and the conditions discussed above.

Although the heat resistance of Salmonella was thoroughly investigated in certain foods, additional data are needed in other products. This review indicated that there are no data yet reported on D-values for Salmonella in fruit juices, despite the fact that some salmonellae outbreaks occurred in orange juice and apple cider (17). Although there are recommendations for inactivating Salmonella in chicken during cooking, only one report presented D-values for Salmonella in chicken meat (110), and the information in that report is insufficient to establish a process for chicken. Salmonella in nearshore harvest water may contaminate raw fish, particularly aquacultured fish (49), and no heat resistance information is available in these types of products.

REFERENCES


Haldidsson, R. A., S. Doens, and R. C. Anantheswaran. 1994. Pa-


THERMAL RESISTANCE OF SALMONELLA


