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# Heat Resistance of *Salmonella* in Various Egg Products

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The heat-resistance characteristics of *Salmonella typhimurium* Tm-1, a reference strain in the stationary phase of growth, were determined at several temperatures in the major types of products produced by the egg industry. The time required to kill 90% of the population (D value) at a given temperature in specific egg products was as follows: at 60 C (140 F), D = 0.27 min for whole egg; D = 0.60 min for whole egg plus 10% sucrose; D = 1.0 min for fortified whole egg; D = 0.20 min for egg white (*p*H 7.3), stabilized with aluminum; D = 0.40 min for egg yolk; D = 4.0 min for egg yolk plus 10% sucrose; D = 5.1 min for egg yolk plus 10% NaCl; D = 1.0 min for scrambled egg mix; at 55 C (131 F), D = 0.55 min for egg white (*p*H 9.2); D = 1.2 min for egg white (*p*H 9.2) plus 10% sucrose. The average Z value (number of degrees, either centigrade or fahrenheit, for a thermal destruction time curve to traverse one logarithmic cycle) was 4.6 C (8.3 F) with a range from 4.2 to 5.3 C. Supplementation with 10% sucrose appeared to have a severalfold greater effect on the heat stabilization of egg white proteins than on *S. typhimurium* Tm-1. This information should be of value in the formulation of heat treatments to insure that all egg products be free of viable salmonellae.

The egg products industry must provide egg products free of viable salmonellae. Various methods have been recommended to achieve this goal. The most efficient processes used involve the application of heat. The treatment of liquid whole egg at 140 F for 3.5 min as a means of killing salmonellae was recommended over 15 years ago (8) and has had successful application for these many years. Recent advances in methods of pasteurizing egg whites now allow the industry to guarantee egg whites free of viable salmonella (10, 15; H. Lineweaver and F. E. Cunningham, U.S. Patent 3,251,697, 1966).

The egg processing industry, however, produces other egg products modified in many ways, such as *p*H adjustment, supplementation with either carbohydrates or salts, separated whites, separated yolks, and fortified whole eggs, to mention the more important. The heat pasteurization treatments mentioned above for whole eggs and whites can be applied to these products, but their effectiveness in insuring a salmonella-free product will vary markedly with the modification used to produce the wide variety of egg products used in the food industry.

Although reports have appeared relating to the heat resistance of salmonellae in egg products (see Table 1), no study has examined all of the major types of egg products produced by the industry. Therefore, this study was undertaken to determine and compare the heat resistance of salmonellae in these various egg products. The decimal reduction time (time to kill 90% of the population) of a standard reference strain of *Salmonella* (13) (i.e., *S. typhimurium* Tm-1), well into the stationary growth phase, was determined in the various egg products and at various temperatures.

Such information should be of value in the recommendation of guidelines for heat treatments necessary to insure that the many egg products produced will receive heat treatments yielding kills of salmonellae equivalent to the treatment used successfully on whole eggs for the past 15 years (8).

## MATERIALS AND METHODS

**Test organism.** *S. typhimurium* Tm-1, a reference strain in this laboratory, with heat resistance characteristics that are comparable to those of other salmonellae (13), was used for this study.

**Cell suspension.** This was prepared, as previously described (7), from a culture well into the stationary growth phase, which provides cells of maximum heat resistance (17).

**Treatment of eggs prior to breaking.** Eggs approximately 1 week old were washed in a detergent sanitizer solution, immersed momentarily in 70% ethyl alcohol to kill the microorganisms on the shell, and then allowed to air-dry. They were then broken out and, when necessary, the yolks and whites were aseptically separated on a sterile, commercial, hand egg separator. The separated white was allowed to fall into a sterile container. The yolks were also combined in a sterile container. The whole eggs or the separated fractions were blended. Various sterile supplements were added to whole egg or to its separate components to yield the desired egg product. Egg products were defined as follows: (i) whole egg, natural composition; (ii) whole egg plus 10% sucrose; (iii) fortified whole egg, 65% whole egg, 25% yolk, and 10% corn syrup (83% solids and 61 to 65 dextrose equivalents); (iv) egg white at its normal *p*H of 9.0 to 9.2; (v) egg white (*p*H 9.2) plus 10% sucrose; (vi) egg white stabilized with lactic acid and aluminum (H. Lineweaver and F. E. Cunningham, U.S. Patent 3,251, 697, 1966); (vii) yolk equivalent to the commercial product (i.e., diluted with egg white to approximately 43% egg solids); (viii) yolk plus 10% sucrose; (ix) yolk plus 10% NaCl; (x) scrambled egg mix (E. Jones et al., U.S. Patent 3,093,487, 1963.

**Determination of heat resistance.** The egg product was inoculated with the salmonella suspension described above to give a final population of approximately $10^7$ per ml. The inoculated product was dispensed in 2-ml samples in 5-ml color-break- (Owens-Illinois Glass Co., Toledo, Ohio), which were then sealed and cooled to ~0 C in an ice-water bath. The sealed ampoules were completely immersed in a 10-gal water bath, the temperature of which was controlled to ±0.05 C. After various periods of exposure an ampoule was removed from the bath and the contents were cooled immediately in an ice-water bath. Appropriate dilutions of the treated sample were spread-plated on Trypticase (BBL) soy plus 2% yeast extract-agar plates. After incubation at 35 C for 24 or 48 hr, or both, colonies were counted.

**Data analysis.** The logarithm of the number of survivors was plotted against the period of exposure to determine the D value at each temperature. The slope of the line over a time interval necessary to result in at least a $10^4$ kill, which occurred after the test temperature had been reached, was used to determine all D values. The logarithm of the D values so determined was then plotted versus temperature (C) to give thermal destruction time (TDT) curves. The slope of the line drawn through these points is equivalent to Z, the temperature interval necessary for D to change by a factor of 10 (2).

**Stabilization of egg-white proteins by 10% sucrose.** Egg white (*p*H 9.2) and egg white (*p*H 9.2) plus 10% sucrose (8-ml samples) were heat treated for various time periods at 60 ± 0.05 C, as described for the heat resistance study, but in 10-ml sealed ampoules. After heat treatment, the relative changes in turbidity or coagulation, or both, in the supplemented and unsupplemented samples were recorded by means of photography.

## RESULTS

A typical plot of the log of the survivors versus the time of exposure for *S. typhimurium* in egg products [egg white (*p*H 9.2) at 52 C] is given in Fig. 1. The time interval between 4.4 and 7.3 min is used as the D value for this experiment. It should be noted that this is the average D involving at least a 4-log kill.

The plotting of D values so determined for egg white and modified egg whites versus temperature (C) is shown in Fig. 2. Similar plots for whole egg and supplemented whole egg are shown in Fig. 3; those for yolk and yolk products are given in Fig. 4. The changes in turbidity or coagulation, or both, of egg white and sugared egg white subjected to various heating periods at 60 C are given in Fig. 5.

From the D values so determined, the relationship between time and temperature required to result in approximately equal pasteurization effectiveness in all the egg products examined is given in Fig. 6.

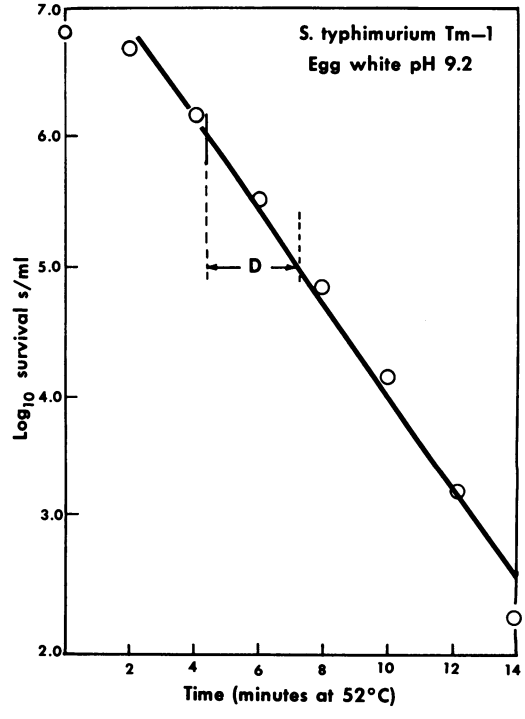


FIG. 1. *Typical plot of the log of survivors versus time of exposure at 52 C for S. typhimurium Tm-1 in egg white at pH 9.2.*

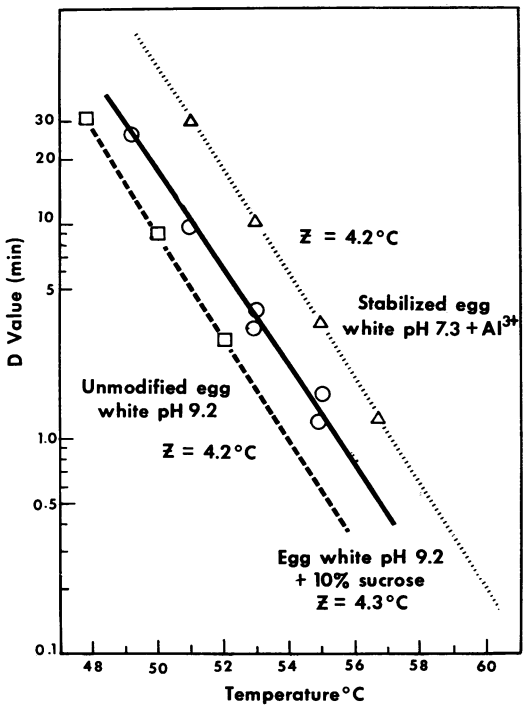


FIG. 2. *Thermal destruction time curves for S. typhimurium Tm-1 in egg white and egg white products.*

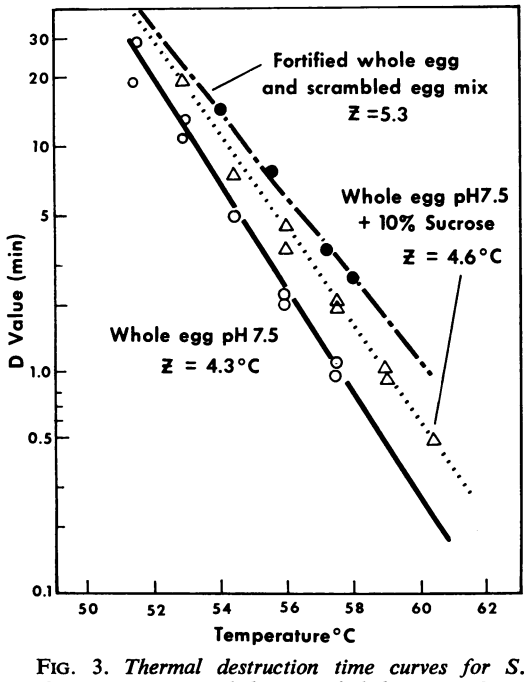


FIG. 3. *Thermal destruction time curves for S. typhimurium Tm-1 in whole egg and whole egg products.*

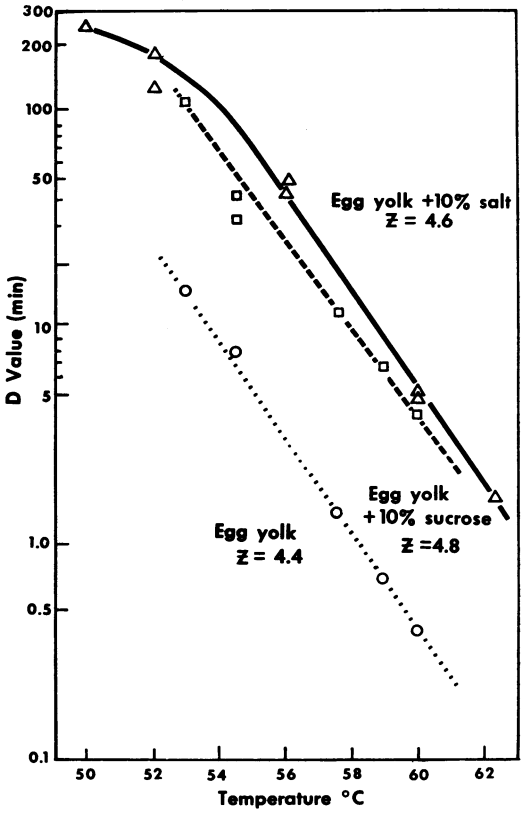


FIG. 4. *Thermal destruction time curves for S. typhimurium Tm-1 in yolk and yolk products.*

## DISCUSSION

The D values for egg white, stabilized egg white (*p*H 7.3 plus Al), whole egg, and plain yolk determined in this study agree very well with those reported by other workers (Table 1). The slight deviations observed may be due to the differences in heat resistance among the many strains of *Salmonella* used (13).

It is also of value to compare our average for Z (4.6 C) with that of Licciardello et al. (12) (4.3 C) for yolk and with that of Lategan and Vaughn (11) for whole egg (4.2 C) at *p*H 5.5. Our Z values for all egg products are grouped in the range of 4.2 to 5.3 C (Fig. 2–4).

The magnitude of the effect of 10% sucrose supplementation of egg white and whole egg on D value of salmonellae is similar to the carbohydrate effect observed by Fay on *Escherichia coli* in ice cream mix (6), i.e., a doubling of the D value in the carbohydrate-containing product.

However, the change in D value in yolk brought about by either 10% sucrose or 10%

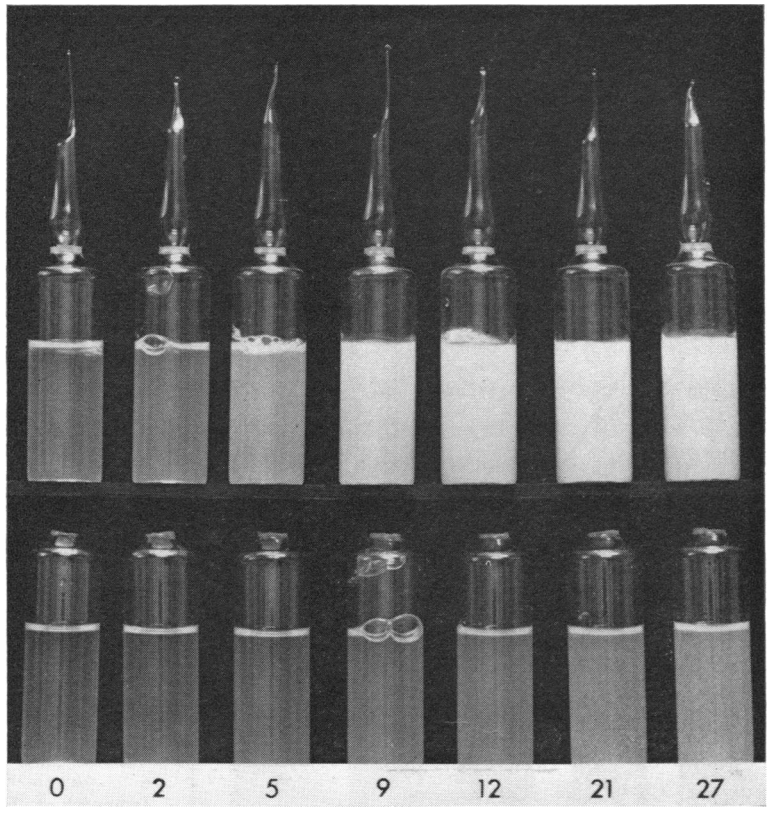


FIG. 5. *Changes in turbidity or coagulation, or both, of egg white and egg white plus 10% sucrose after exposure to 60 C. Numbers signify minutes at 60 C. Top row is unsupplemented egg white; bottom row is egg white plus 10% sucrose.*

NaCl supplementation is quite dramatic. Such an increase in heat resistance (from D to 10 D) indicates that at temperatures where $10^{10}$ cells/ml are killed in 1 min in yolk, only 10 cells/ml will be killed in yolk supplemented with either 10% salt or 10% sucrose. This, to our knowledge, is the first time that such stabilization to heat of bacterial vegetative cells by sodium chloride has been observed. However, a slight effect (D of 14 min versus D of 19 min) on the heat resistance of spores was reported when pea liquor was supplemented with 10% salt (19). Results of experiments in our laboratory indicate that salt has no protective effect on salmonella heated in a buffer system. Similar effects on D values by sucrose have been noted at concentrations of 30% or greater (3).

From the data given in Fig. 2, 3, and 4, it is obvious that the heat sensitivity of salmonella varies widely in the various products produced by the egg products industry. Past experience has indicated that the treatment recommended for pasteurizing whole egg (i.e., 140 F for 3.5 min) is sufficient to insure a viable salmonella-free product. Therefore, it has been chosen as the reference treatment. Treatments for other egg products corresponding to this reference treatment can easily be determined from the summation of our data (Fig. 6). For egg white, pasteurization-equivalent treatments may be determined by selecting the plot for this product and reading the temperature on the abscissa and the holding time on the ordinate. That is, for a holding time of 3.5 min it is necessary to maintain *p*H 9.2 egg white at a temperature of not less than 133.2 F; similarly for yolk, 3.5 min at 141.1 F, for egg white *p*H 9.2 plus 10% sucrose, 3.5 min at 135.9 F; etc. On the other hand, if one selects a given temperature for treatment (abscissa), the corresponding holding time is given on the ordinate; i.e., for stabilized egg at 138 F a holding time of approximately 4 min is needed; for whole egg plus 10% sucrose at 138 F, 11 min is necessary. Of course, a time-tempera-

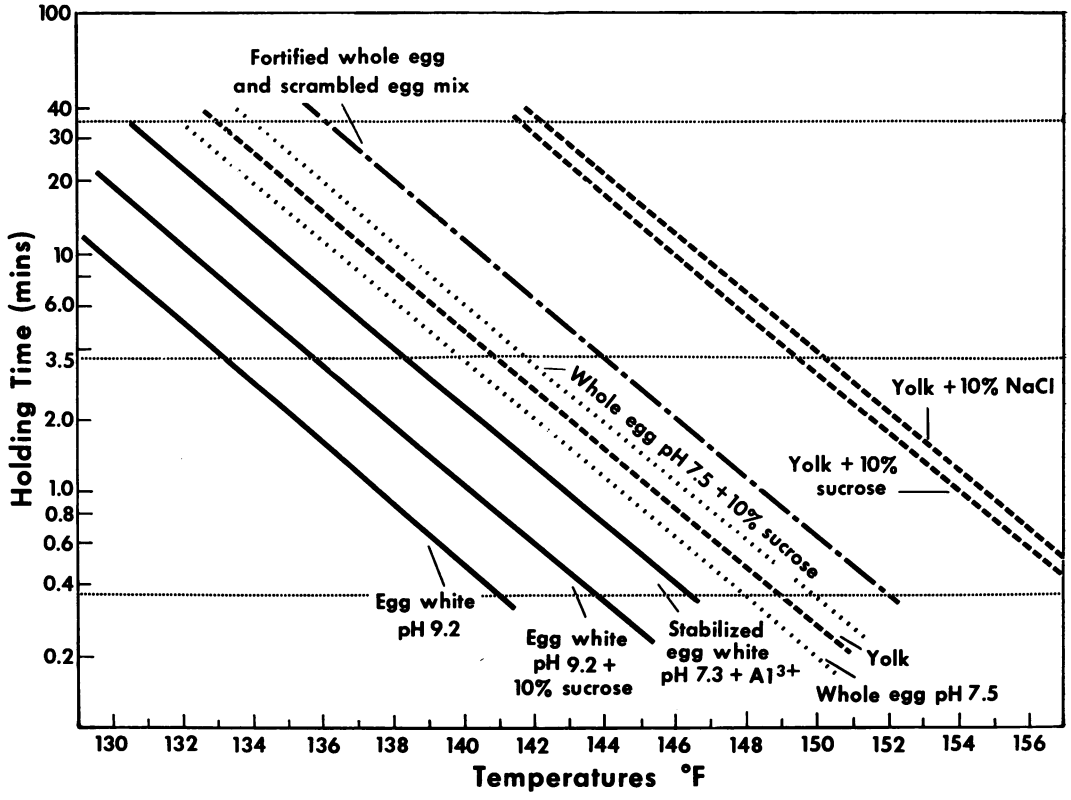


FIG. 6. *Composite thermal destruction curve for S. typhimurium Tm-1 for all egg products examined.*

ture relationship convenient to the operating equipment and compatible with the stability of the egg product must be chosen.

Treatments equivalent to the standard (3.5 min, 140 F for whole egg) afford a wide margin of safety. Such treatments insure a kill in excess of $10^{12}$ cells/ml of *S. typhimurium* Tm-1; D = 0.27 min at 140 F, the reference strain used in this study. If a strain with twice the heat resistance is encountered, a $10^6$ kill would still result. Since the physiological state of the organism used was chosen to give cells of maximal heat resistance, an additional safety factor has been incorporated; that is, the D value of the stationary growth phase cells used is severalfold that of logarithmic-phase cells (17). Although these calculations assume a full treatment time of 3.5 min, it is recognized that under commercial pasteurizing conditions the minimal holding time may be 60 to 80% of the average holding time (9).

Although the heat resistance of salmonellae is doubled when *p*H 9.2 egg white is supplemented with 10% sucrose, it appears that the egg white proteins are heat stabilized to a greater extent (Fig. 5). The unsupplemented egg white becomes somewhat turbid after 2 min at 60 C and progresses rapidly to heavy coagulation after 9 min at this temperature. In contrast, egg white supplemented with 10% sucrose is only slightly turbid after exposure for 21 min at 60 C, and no coagulation is evident even after 27 min at this temperature. This stabilization effect has been noted previously (4, 16).

Egg white supplemented with 10% sucrose may, therefore, be heated to higher temperatures, giving greater kills of salmonellae than those obtained in plain egg white, without alteration of the functional properties of the egg white proteins. Additionally, as has been shown by Kline et al. (U.S. Patent 3,170,804, 1965), egg white with added sucrose gives a spray-dried product with storage stability characteristics equivalent to those of the spray-dried, desugared product. In other words, it may be possible to produce a spray-dried product which has been pasteurized, but not desugared, with functional and storage stability properties equivalent to those of the present product.

TABLE 1. *Comparison of D values with those of previous workers*

| Product and temperature | Present study | Previous workers[a] | Reference |
|---|---|---|---|
| Egg white, *p*H 9.2; 54.8 C | 0.64 | 0.94 | 5 |
| Egg white, *p*H 9.2; 134 F | 0.25 | 0.54 | 10 |
| Egg white, *p*H 7.3 Al; 58.3 C | 0.54 | | |
| Egg white, *p*H 7.0 Al; 58.3 C | | 0.94 | 5 |
| Whole egg, *p*H 7.5; 55 C | 4.0 | 3.0 | 11 |
| Whole egg, *p*H 7.5; 140 F | 0.28 | 0.25 | 18 |
| Whole egg, *p*H 8.0; 140 F | | 0.29–0.62 | 1 |
| Whole egg, *p*H 7.6; 148 F | | 0.43 | 1 |
| Whole egg; 138 F | 0.5 | 0.28–0.62 | 14 |
| Yolk; 140 F | 0.4 | 0.2–0.5 | 12 |
| Yolk; 138 F | 0.7 | 0.92 | 14 |

[a] As given by the authors or calculated from their data.

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