Effect of Added Moisture on the Heat Resistance of *Salmonella anatum* in Milk Chocolate

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Received for publication 5 September 1969

The heat resistance of *Salmonella anatum* in milk chocolate at a processing temperature (71 C) was greatly decreased by adding 1 to 4% moisture.

Chocolate is considered an unlikely food for microbial growth because of its low moisture and high content of sugar and fat; moreover, it undergoes a heating process during its manufacture that greatly limits the number of organisms present.

A critical factor in the heat resistance of salmonellae is the amount of available moisture in the food product. It has been reported (2) that the heat resistance of salmonellae in dried egg white is 600 to 700 times higher than in liquid egg whites. Ingredients such as sucrose can act to remove available water. Foster (1) reported at least a 10-fold increase in the heat resistance of *Salmonella typhimurium* when the sucrose concentration was increased from 5 to 40% at 60 C. Since one of the major differences between milk chocolate and other food products is the low moisture content, this study was made to determine the effect of additional moisture on the heat resistance of salmonellae in milk chocolate at a practicable processing temperature. At the temperature chosen for this study, 71 C, the physical properties of milk chocolate are not seriously altered.

*Salmonella* cultures isolated from chocolate products were obtained from the Food and Drug Administration. *S. anatum* (FDA no. 3989) was found to be the most heat-resistant strain in milk chocolate of our collection; therefore, it was used in this study.

To avoid altering the moisture content of the chocolate and to prevent osmotic shock of the cells upon inoculation, lyophilized cultures were used for inoculation. Lyophilization was accomplished by centrifuging 500 ml of a 25-hr nutrient broth culture, resuspending the cells in 40 ml of sterile skim milk, freezing, and drying the cells in a Stokes 21 F O Freeze Dryer.

The level of contamination has been uniformly low among the few samples of milk chocolate found positive in respect to salmonellae. To simulate these low levels, large quantities of milk chocolate [17 lb (7.7 kg) per batch] were inoculated to give less than 20 cells per 100 g of chocolate. The chocolate was then heated to 71 C in an electrically powered, water-jacketed, swept-surface tempering kettle [40-lb (18 kg) capacity].

Moisture in amounts of distilled water, ranging from 1 to 10% (w/w), was added to the heated milk chocolate as an aerosol mist or droplets.

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1 Authorized for publication on 28 July 1969 as paper no. 3636 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
With constant agitation, the lyophilized culture was added to the milk chocolate-water mixture.

At periodic time intervals, chocolate samples were withdrawn, and the number of surviving salmonellae was determined by the three-tube most-probable-number technique. Nutrient broth was used as the recovery medium. Suspect colonies were confirmed biochemically and serologically.

The log₁₀ of the number of surviving cells was plotted as a function of time. The survivor curves were extrapolated to obtain the $D$ value (time required to reduce the population by 1 log cycle). The $D$ value was then plotted as a function of percentage of added moisture. The effect of added moisture on the $D$ value of *S. anatum* in milk chocolate at 71°C is shown in Fig. 1. A dramatic decrease in the $D$ value was evidenced with 2.0% added moisture, reducing the $D$ value from 20.0 hr to 4.0 hr. $D$ values decreased as the level of added moisture increased. However, as illustrated in Fig. 1, the change per increment of moisture was especially pronounced at the 2.0% level and below. In no instances were survivors detected after heating periods longer than the calculated $D$ value with low initial inocula.

With high initial inocula ($10^6$ cells per g of chocolate), when the chocolate was heated for periods of time greater than that calculated to assure a salmonellae-free product, viable cells were detected. A progressive loss of moisture occurred when the chocolate was heated, with the greatest loss occurring early in the heating period. For example, with 2.0% added moisture, the moisture was reduced from 3.72 to 2.50% during 2 hr of heating at 71°C, as shown in Table 1. This moisture loss may be offered as a possible explanation as to why survivor curves with large initial inocula showed a “tailing off” effect.

Since only one strain of *Salmonella* was employed in this study, caution should be used in applying these results to all situations involving contamination of milk chocolate. With low levels of contamination, the results indicated that a short-time heating process with additional moisture could be used for recovering milk chocolate contaminated with salmonellae. By continuously replacing moisture, it might be possible to render chocolate salmonellae-free, even with relatively high levels of contamination.

This investigation was sponsored by the Chocolate Manufacturers' Association of the U.S.A.

**LITERATURE CITED**

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2. Prost, E., and H. Riemann. 1967. Food borne salmonellosis. *Annu. Rev. Microbiol.* 31:500.