Growth of *Salmonella typhimurium* in Skim Milk Concentrates

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The influence of various levels of skim milk solids and temperature on the duration of lag phase, growth rate, and extent of growth of *Salmonella typhimurium* was investigated. The effect on growth of salmonellae (and a strain of *Escherichia coli*) of reduced pressure at a constant solids level and under conditions simulating vacuum condensation of skim milk was also studied. *S. typhimurium* grew when inoculated into skim milk solutions ranging from 10 to 60% solids and over a temperature range of 23 to 44 C. At 10 to 12 C, growth was evident only in the 10% skim milk. As the total solids level was increased or incubation temperature was deviated from the optimum, or both, there was an increase in the lag phase and generation time of salmonellae. A lower cell population also resulted. The generation time at 37 C of *S. typhimurium* incubated at atmospheric pressure was approximately one-half that in skim milk concentrates held under reduced pressure. In addition, a slightly longer lag phase and lower cell yield characterized the growth under reduced pressure. Concentration of skim milk had little or no effect on viability of salmonellae or *E. coli* when the vapor temperature in the vacuum pan was below the maximum growth temperature for salmonellae. Increasing the vapor temperature to 48 C caused a two-log reduction in viable organisms during the concentrating period (65 min).

The genus *Salmonella* has received increased notoriety in recent years, prompted in part by recovery of these organisms from dried milk implicated in a 1965 outbreak of *Salmonella* food poisoning (3). Subsequent investigation disclosed that various dried dairy products were contaminated with salmonellae.

Although various constructive opinions (4, 5, 7) have been given regarding probable sources of salmonellae and suitable conditions conducive for growth of these organisms in processing plants, the literature contains little information on the actual behavior of salmonellae during the manufacture of nonfat dry milk (NDM).

It is becoming increasingly evident that the behavior exhibited by salmonellae (and other microorganisms) in laboratory media is not necessarily characteristic of the organisms in a food environment. The basis for this discrepancy is in part attributable to the physicochemical properties of the given food substrate and in part to the influence of other microorganisms constituting the normal flora of the product. Consequently, to be certain how salmonellae will behave in a given product or process environment, experimentation must be conducted using the food in question and simulating the processing this food may receive.

This study was conducted to generate data on the behavior of salmonellae during the manufacture of dried milk products.

**MATERIALS AND METHODS**

**Bacterial cultures.** The strains of *S. typhimurium* and *Escherichia coli* used in this study were obtained from the Food Research Institute culture collection. Stock cultures were maintained on nutrient agar slants at room temperature. Working cultures were transferred daily in Trypticase soy broth (TSB) and incubated without agitation at 32 C unless otherwise noted.

**Skim milk.** The milk was prepared from a single lot of antibiotic-free skim milk, which was spray-dried in the dairy plant facilities of the University of Wisconsin. The NDM met the following specifications: no detectable coliform or *Salmonella* microor-
ganisms when tested by the procedures advocated by the Food and Drug Administration (1), a standard aerobic plate count of less than 300 per g, and a moisture content of approximately 2.5%.

Growth at atmospheric pressure. A 12-hr culture of _S. typhimurium_ was added at a 0.5 to 1.0% (v/w) level to milk solutions, which were at 10, 30, 40, 50, and 60% (w/w) total solids (TS) levels. Concentrates were prepared in Nalgene containers by adding the appropriate amount of NDM to sterile distilled water which had been tempered to the test incubation temperature, i.e., 10 to 12, 23, 32, 37, and 44 C. A mechanical blending apparatus was used to mix thoroughly the inoculum into the milk concentrate. The inoculated concentrates were then incubated at the test temperature to which they have been pretempered.

Growth under reduced pressure. Reduced pressure growth studies were conducted at 35 to 37 C with a 40% (w/w) milk concentrate, which was contained in a laboratory vacuum pan that had been modified for bacterial quantitation by the incorporation of a sampling port into the system (3a).

To simulate vacuum condensing conditions, the laboratory vacuum pan including an external heating source (steam) was employed. Concentration of 10% skim milk to higher TS levels was carried out by matching the rate of inflow of 10% skim milk to the outflow rate of condensate. Material balance calculations were used to determine the TS level attained per volume of 10% skim milk added (volume of fluid in the system constant) at the desired vapor temperature. The inoculum was added into and samples were taken from the vacuum pan as described previously (3a). A manometer and a thermometer were employed to monitor the pressure and vapor temperature during the concentration process.

Enumeration of salmonellae. Quantitation of _S. typhimurium_ in the growth experiments was accomplished by periodically transferring a 10-g sample to a sterile, chilled Waring Blender containing 90 ml of sterile distilled water. After blending for approximately 1 min at low speed, 0.1-ml samples of the appropriate dilutions were surface-plated on Salmonella-Shigella agar (BBL). These plates were examined for typical Salmonella colonies after incubation at 35 to 37 C for 48 hr.

Enumeration of salmonellae during the vacuum concentration of milk was performed by periodically removing 1-ml samples to 9 ml of 0.1% peptone water. A 0.1-ml amount of the appropriate subsequent dilutions were surface-plated on Trypticase soy agar (BBL) fortified with 0.2% yeast extract (TSAYE). The plates were examined after incubation at 35 to 37 C for 48 hr.

Enumeration of _E. coli_. Enumeration of _E. coli_ in the growth experiments was accomplished by making pour plates with violet-red bile-agar of the appropriate dilutions of concentrate. Plates were examined after 18 to 24 hr at 35 to 37 C. Enumeration of _E. coli_ during the vacuum-condensing operation was performed in an identical manner as that described above for salmonellae.

RESULTS AND DISCUSSION

Growth at atmospheric pressure. The initial approach was to study the effect of various incubation temperatures and TS levels on the growth of _S. typhimurium_ at atmospheric pressure. Although the NDM used in the concentrate preparation was high-quality powder, it was not a sterile product. This necessitated the employment of a selective (and differential) medium for the quantitation of viable salmonellae throughout the incubation period. Salmonella-Shigella agar was chosen as the recovery medium after preliminary experiments disclosed that the interfering organisms were primarily _Bacillus_ spp. which grew well and masked typical _Salmonella_ colonies on Brilliant Green-agar. The inoculum was at a level to insure detection of salmonellae by the surface-plating procedures throughout the experimental period.

The growth curves of _S. typhimurium_ in milk solutions (10 to 60% TS) incubated at several temperatures are shown in Fig. 1 (a–e). Figure 2 depicts the growth pattern of _S. typhimurium_ at a single TS level (40%) as the growth was influenced by incubation temperature.

In all trials, there was a loss of recoverable salmonellae upon introduction into the milk solutions. In solutions containing higher TS levels, most of the loss was manifested during the time (5 min) that elapsed between introducing the inoculum and taking the initial sample. At the lower TS levels, the loss was not as great during this time, but there was a continued gradual loss of recoverable cells for several hours thereafter, the rate being dependent on the temperature of the concentrate.

It is quite probable that the major adverse effect on the cells was the abrupt change in osmotic pressure. The more drastic the change, the more rapid the loss of recoverable salmonellae. The simultaneous change in temperature could also be seen to contribute to the demise of salmonellae as is indicated when the curves obtained at a single TS level but different incubation temperatures are examined (Fig. 2). However, this effect was slight in comparison to the osmotic pressure influence. Since the data depicted in these figures were derived from counts made on Salmonella-Shigella agar, these points represent the number of salmonellae recoverable on this agar. Parallel experiments in which TSAYE was used as a recovery medium (until overgrowth by the
normal flora made enumeration of salmonellae impossible) were run. The same pattern of behavior was obtained, i.e., a die-off of salmonellae in the concentrates followed by an "adjustment period" before the number of replicating cells exceeded the number of cells that were dying. However, the loss in recoverable salmonellae upon inoculation as measured on TSAYE did not exceed 0.5 log in any of the milk concentrates. Moreover, the adjustment period was shorter when TSAYE agar was used. It is thus apparent that the osmotic stress imposed by inoculation into concentrated milk rendered the cells less able to cope with the rather adverse environment of a selective agar medium. This is not unexpected since the work of others (2, 8) has repeatedly demonstrated that injured or stressed cells are physiologically debilitated and are more susceptible to the harsh environment of selective recovery media.

The loss of recoverable salmonellae upon inoculation into concentrated milk solutions underscores the importance of performing counts on the inoculated material rather than basing the zero time cell count on an enumeration of the organisms in the cell suspension used as the source of inoculum. Unless this initial count is made, the time at which actively growing cells constitute the major portion of the population and the curve begins to approach a logarithmic nature may be missed by several hours. For example, the data of McDonough and Hargrove (9) would indicate that a lag phase of slightly more than 24 hr occurred before salmonellae were able to prolif-
erate in a 60% TS concentrate at 37°C. Our results show that, although the most rapid growth began at 15 hr, the population of S. typhimurium began to increase 3 hr after inoculation into 60% TS at 37°C. Thus, these cells would manifest the characteristics (e.g., sensitivity to chemical and physical agents) of log-phase cells rather than lag-phase organisms. Whether this difference in sensitivity is sufficient to have a bearing on the survival by salmonellae of in-plant processing is questionable.

The mean generation times for S. typhimurium in milk concentrates are given in Table 1. Increasing TS concentrations and lower incubation temperatures both resulted in slower growth by salmonellae. Similar results were reported by Wodzinski and Frazier (10) in their study of the effect of solute concentration and incubation temperatures on three species of bacteria.

It should also be noted that increased concentrations of solids also resulted in a lower cell yield of salmonellae. This effect was particularly in evidence for the 50 and 60% TS solutions incubated at and above 32°C. Although this may be of interest to the researcher, it is rather unimportant to the producer since significant levels of salmonellae were achieved in all concentrates stored at or above 23°C. At 10 to 12°C, salmonellae were able to proliferate only in the 10% TS solution, and a reduction in viable salmonellae was noted in all other solutions. Most of the die-off was completed within 72 hr, and the population remained relatively stable thereafter. Thus, refrigeration of milk concentrates will not only prevent growth of salmonellae but also result in a reduction in viable cells. However, it would not be prudent to assume that such practices would ensure a Salmonella-free concentrate.

Growth under reduced pressure. Experiments designed to generate data on the behavior of salmonellae under reduced pressure were undertaken to determine the conditions that would permit the proliferation of salmonellae during the vacuum concentration of skim milk. For comparative purposes, a strain of E. coli was also included. Both organisms were inoculated (in separate experiments) into 40% TS milk solutions and incubated at 35 to 37°C and 55 ± 5 mm of Hg pressure. Under these conditions, the mean generation times were 0.8 and 1.0 hr for E. coli and S. typhimurium, respectively. The more rapid growth of E. coli was most probably due to its ability to ferment lactose, a characteristic lacking in S. typhimurium. It should be noted that the generation time at 37°C of S. typhimurium in the 40% TS solution at atmospheric pressure was approximately one-half as long as under reduced pressure. Anaerobic growth of salmonellae in skim milk is possible by the energy-generating arginine dihydrolase system that these organisms have. However, this system would not be as efficient as an oxidative amino acid metabolism. This would be a possible explanation for the difference in growth rates of salmonellae in the aerobic and anaerobic environments. In addition to a slower growth rate,
the culture grown under reduced pressure achieved a lower final population than the culture grown at atmospheric pressure. This latter observation is in accord with that of George et al. (6) on the growth of S. aureus in skim milk concentrates incubated under reduced pressure. These investigators concluded that the subatmospheric pressures used in vacuum concentration would not afford sufficient retardation of growth to be of practical significance. Based on our observations, we would agree that this premise is also true for control of salmonellae during vacuum concentration of milk.

The effect of vacuum concentration of skim milk from 10 to 42% TS on the behavior of E. coli and S. typhimurium was investigated. Multiple trials employing the pressure-temperature parameters summarized in Table 2 were conducted. The results of a typical single trial with each organism are shown in Fig. 3 and Fig. 4. It is evident that no significant decrease in cell number occurred when the vapor temperature was below the maximum growth temperature for salmonellae. At temperatures slightly above 46°C, there was approximately a 2-log decrease in viable cells during the concentration process. It is probable that the short time (47 to 56 min) necessary to accomplish the concentration precluded growth of the organisms when the temperatures were below the maximum growth temperature. It is quite probable that given sufficient time to adjust to the environment, these organisms would multiply in the concentrate as was described above.

This work and that of McDonough and Hargrove (9) have amply demonstrated that salmonellae will grow quite readily in milk solutions (up to 60% TS) if the temperature is appropriate. The application of reduced pressure to milk solutions to effect a concentration of

| Vapor temp | Manometer reading (cm of Hg) | Condensing time (min) |
|------------|------------------------------|-----------------------|
| C          | F                            |
| 33.0       | 91.4                         | 3.6-3.7               | 47         |
| 41.6       | 106.9                        | 5.5-5.7               | 56         |
| 48.0       | 118.2                        | 7.4-7.6               | 65         |

**Table 2. Conditions existing during vacuum concentration (from 10 to 42% total solids) of artificially contaminated skim milk**

*Fig. 3. Influence of vapor temperature on the survival of Salmonella typhimurium during vacuum concentration of milk.*

*Fig. 4. Influence of vapor temperature on the survival of Escherichia coli during vacuum concentration of milk.*
solids does reduce the growth rate of salmonellae, but the process will not kill the cells if the temperature is below the maximum growth temperature for salmonellae. The processor should now realize that if salmonellae do gain entry to pasteurized milk, the surest method of preventing increases in cell number during further processing, i.e., vacuum concentration, is strict temperature control. Product cannot be left within the growth temperature range for salmonellae for any significant period of time if the population of the contaminant is to be maintained at a level low enough to be destroyed during subsequent drying treatments.

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