Survival of *Lactobacillus acidophilus* in a Spray-Drying Process

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ABSTRACT

A frozen concentrate of *Lactobacillus acidophilus* was used as inoculum for milk solids-not-fat (MSNF) reconstituted to 25 and 40% solids. Initial count of the two milks was $1.2 \times 10^8$ and $7.0 \times 10^8$ CFU/g of solids, respectively. Sublots of these two concentrates were spray-dried at 85-, 80-, and 75-C exit air temperature in a Coulter/Townley pilot dryer (vertical, venturi nozzle spray system). Survival of *L. acidophilus* was greatest at the lowest outlet air temperature investigated, and in the milk of lower solids content. At 75-C exit air temperature the count following drying was $2.6 \times 10^7$ per gram of solids at 40 percent solids, and $9.8 \times 10^6$ per gram of solids at 25% solids. Percent survival after 30 days storage under nitrogen at 4 C was 1.29 and 4.17, respectively, for the two solids levels.

Evidence is increasing regarding the potential of *Lactobacillus acidophilus* to ameliorate a number of digestive upsets (4,5,10,11,12,14). While the data are inconclusive, the organism in sufficient numbers appears to have the ability to aid lactose digestion among persons known to suffer a degree of lactose intolerance. While the latter problem appears not so severe as to restrict milk intake altogether, it causes various degrees of discomfort when sizeable amounts of milk are ingested on an empty stomach. Most of the world's population, Latin Americans and Asians included, are sensitive to this nutritional ailment. Around the world, in rich and poor nations alike, other digestive problems occur as a result of poor nutrition, some of which this organism might very well alleviate if ingested in regular amounts on a daily basis. For these and other reasons, "Sweet Acidophilus" milk is now marketed throughout most of the U.S. Current information suggests that an intake of about one billion viable cells of the organism daily will serve the maintenance requirements in the human body (6,13). To achieve this level of intake on a practical basis requires some 2-7 million viable cells per ml of fluid milk. At least this is the regulatory standard now being considered (8).

Obviously a fluid milk product has limited shelf life and is not readily transportable to distant markets at low cost. A dry product could better serve these needs, if sufficient numbers of *L. acidophilus* could survive both spray-drying and storage. In Chile, such a product could serve as an especially useful purpose, in that milk production is localized in the southern part of the country with much of the population located between 1,000 and 2,000 kilometers north of the supply area. Under such conditions, and for transportation over long distances, a dry product containing viable *L. acidophilus* cells could be processed in the milk supply area, then shipped to consumption centers, either to be reconstituted into a Sweet Acidophilus - type milk and sold on a "fresh" liquid basis, or for consumer reconstitution of a packaged dry product.

The main question at issue is survival of the organism during spray drying and storage in numbers and in such viable condition as to make possible the maintenance level of intake through a milk product. Apparently, work in this area has been discouraged, generally because of evidence of poor survival of *L. acidophilus* in the dry state at room temperature (2). It may likewise have been discouraged by past assumptions that very large numbers of organisms were necessary to achieve an appropriate ratio of *L. acidophilus* to other intestinal microorganisms. Smaller numbers do now appear to serve the purpose adequately (13), if not better. This may make a spray-drying process more feasible than was formerly thought. In any event, evidence of the growing importance of this organism to the human diet appeared to the authors to offer sufficient justification to determine survival during spray-drying and short-term storage. Storage of the dry product in this study was limited to holding at refrigeration temperature (4 C).

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MATERIALS AND METHODS

Frozen concentrate of *L. acidophilus* was obtained from Miles Laboratories, Inc., Marshall Division, Elkhart, Ind. (46514). This culture is one currently being used in production of Sweet Acidophilus milk. Though not in any way recommended, it was necessary to store the frozen culture three months before undertaking the present study. The temperature of storage was −40 C. No doubt some viable cells were lost during this time but sufficient numbers remained to make possible this investigation.

For ease of processing, two concentrations of milk were prepared for drying purposes by reconstituting nonfat dry milk rather than containing fresh milk. The dry milk was tested and found free of inhibitors. Two lots were made up, at 25 and 40% solids, respectively, on different days. Frozen concentrates of *L. acidophilus* were thawed, and the culture added directly to the concentrated milk. Aseptic conditions of handling the thawed culture were used. Temperature of milk during inoculation was 37 C. Each lot of milk was then subdivided into three sublots for drying in the Coulter/Townley pilot plant dryer of the University. This is a direct-fired, gas-heated, vertical-type dryer, using a spray nozzle and venturi inlet for atomizing the incoming milk. Though smaller than commercial dryers, it is large enough to provide generally equivalent drying conditions. Sublots were dried at a constant inlet temperature (170 C) and three different outlet air temperatures, 75, 80 and 85 C. A constant feed pump was used to pump the inoculated milk into the drying chamber and ultimately to a collector.

Samples of the inoculated milk were taken for microbiological examination before and immediately after drying. Dried samples were also stored either in air or under nitrogen. For the former, small, medium-density polyethylene pouches were used. For the latter, laminated polyester ionomer film (Champion Packages Div., Champion International, Minneapolis, MN) was used. This film has negligible moisture/vapor transmission. Stored samples were all held at 4 C, and examined for viable *L. acidophilus* after 30 days of storage.

The procedures for determining *L. acidophilus* was that given by Miles Laboratories, Inc., Marshall Division, using All Purpose agar with Tween 80 (APT agar). This is a non-selective agar which, for growth of *L. acidophilus*, requires anaerobic conditions and incubation at 37 C for 48 h. The method given by Miles Laboratories, Inc. was modified only in the way in which anaerobic conditions were created. For this work, anaerobic jars were used. Plates were placed in the jars, air removed by vacuum, and the jars flushed with carbon dioxide. Milk samples for *L. acidophilus* analyses were prepared according to Standard Methods for the Examination of Dairy Products (13).

RESULTS AND DISCUSSION

Data in Table 1 show the effect of three different exit air drying temperatures and two different MSNF concentrations on survival of *L. acidophilus*. In all instances, inlet air temperature was constant at 170 C. At both solids concentrations there was an expected (8,9) sharp decrease in numbers of survivors as the outlet air temperature was raised. At 40% MSNF, the numbers of viable organisms decreased from $7.0 \times 10^8$ to $2.6 \times 10^7$ colony forming units (CFU)/g of solids at 75 C and $3.6 \times 10^8$ and $1.8 \times 10^8$ CFU/g of solids at 80 and 85 C, respectively. At these three drying conditions greater survival was noted at 25% MSNF, though part of the increase no doubt was due to the slightly higher initial count. While an attempt was made to standardize the initial count, more than one can of commercial frozen concentrate of *L. acidophilus* was used as inoculum. It is possible that the counts in different cans differed to some extent. Cans had been stored three months before the investigation, and though deep frozen during that time, some loss in viable cells might have occurred. That such loss might have been different in different cans would depend to some extent on whether all cans of concentrate came from the same batch and were of similar age. A 2-h delay in doing the initial counts was also unavoidable. Samples taken from both 40% and 25% solids batches were held refrigerated during this time, but loss may well have taken place to a greater extent in the higher solids batch, mainly due to solids concentration per se. As can be noted in Table 1, pH was nearly the same for both batches. In any event, survival of *L. acidophilus* was higher in the lower solids milk concentrate. For the three outlet air temperatures (85, 80, and 75 C), numbers of survivors were $5.9 \times 10^8$, $2.9 \times 10^7$, and $9.8 \times 10^6$ CFU/g, respectively. Looking at the data in terms of log reductions in count (Log $N_o/N$), it may be seen that the reduction was lower at 25% solids than at 40% solids for any given exit air temperature.

Aside from milk concentration and the possible effect of osmotic pressure on cell survival, drying conditions would also be expected to vary with difference in solids concentration of incoming milk. Given similar inlet pressure and nozzle orifice size, higher solids milk would result in larger particles of spray. As others point out (16), larger particles are subjected to greater heat damage than smaller ones under any given set of drying conditions. Microorganisms entrapped in the particles would also be subjected to that much more heat. Several factors influence cell death or survival. First, as water activity ($a_w$) decreases at the surface of the particle, wet bulb temperatures are exceeded. It is at this point that bacteria may be subjected to killing temperatures. But it is also known that while the bacterial cells are in the intermediate moisture range, they are less sensitive to effects of heat (1,7). Furthermore, the surviving

<table>
<thead>
<tr>
<th>Concentration of skim milk (MSNF) (%)</th>
<th>pH</th>
<th>Drying temperatures (C)</th>
<th>Before drying (CFU/g solids)</th>
<th>After drying (CFU/g solids)</th>
<th>Log reduction of viable cells [Log ($N_o/N$)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air in</td>
<td>Air out</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>6.2</td>
<td>75</td>
<td>80</td>
<td>85</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6 x 10^7</td>
<td>3.6 x 10^8</td>
<td>1.8 x 10^9</td>
<td>9.8 x 10^7</td>
</tr>
<tr>
<td>25</td>
<td>6.3</td>
<td>75</td>
<td>80</td>
<td>85</td>
<td>1.2 x 10^8</td>
</tr>
</tbody>
</table>

1. $\log (N_o/N)$ is taken as the number of viable cells of *L. acidophilus* in one gram of solids in the concentrated milk ($N_o$) divided by the number of viable cells of the organism in one gram of solids of skim milk powder.
organisms, once dried, become somewhat more resistant to heat damage (9). Obviously, such factors for and against survival are interrelated during the drying process. But because large particles dry more slowly than small ones, heat damage to bacterial cells is likely to be more pronounced. Of course, drying conditions can be varied to some extent to produce smaller particles, thus faster drying. This is one factor that should be considered in further work, especially as drying efficiency is directly related to MSNF concentration of the product to be dried.

Though three drying temperatures constitute barely enough points to plot energy of activation ($E_a$) curves, it was thought worthwhile to determine whether or not the data are in reasonable agreement with such values for other organisms. In making the calculation, drying times are considered to be 2 sec. Figure 1 shows the plot of $E_a$ at the two different MSNF concentrations. In both instances, activation energy was lower at higher temperatures, though the difference at 25% solids was slight: i.e., 17.4 Kcal/mole vs 19.9 Kcal/mole. Data on milk at 40% solids were 6.5 Kcal/mole and 23.3 Kcal/mole at higher and lower drying temperatures, respectively. Noting again that only three temperatures were used, there is evidence of a break in the curve, a point at which a new and different $E_a$ is noted. Both the break and the $E_a$ values are consistent with data of Elizondo and Labuza (3) for other organisms. Although the solids concentrations used in this study were different from those used by Elizondo and Labuza, the break in the curve comes at about the same temperature; i.e., 80 vs. 84 C, respectively. Nevertheless, further work over a broader range of temperatures is needed to determine whether or not the break in the curve is a true break or an artifact of these data.

Data in Tables 2 and 3 show percent survival of *L. acidophilus* after 30 days of storage of the dried product at 4 C for the three different outlet air temperatures studied, and for 40 and 25% MSNF levels, respectively. Both survival in air and under nitrogen are indicated. Percent survival is expressed as percent of the original population in the concentrated milk before drying. Figures are given for percentage decrease immediately after drying and after 30 days of storage. Percent moisture in the final product, which varied because of the conditions imposed upon the drying process, ranged from higher to lower as the exit air temperature increased.

Data in both Tables 2 and 3 indicate some slight improvement in survival during storage when the product was held under nitrogen. Nonetheless, good survival, comparatively speaking, was noted in samples stored in air. To some extent, survival in air may have been improved as a result of conditions inherent in the drying system. In operation of the pilot dryer used in the study, much of the oxygen in the air is consumed. Less oxygen would be available, therefore, to cause damage to bacterial cells. Whether this effect might carry over during storage, after air has equilibrated with the powder, is another matter.

Again, major differences in total number of survivors related mainly to concentration of MSNF used in drying. While storage for 1 month resulted in about 50% loss in *L. acidophilus* organisms at any given set of drying conditions, the percentage of survivors was three to four times as great in the product dried at lower than the higher solids content. Moreover, the absolute numbers of survivors dried at 75-C outlet air and stored for 1 month were in excess of the minimal numbers necessary for achieving maintenance levels of intake; this on a dried product reconstituted to 10% solids. It remains to be determined, however, whether the organisms are still capable of growth under conditions existing in the intestinal tract. To test this potential, it would be necessary to attempt to grow the organisms under those conditions recently cited by Speck (13) for determining bile resistance. Obviously, much work remains to be done, both in examining the most suitable drying technology and determining the most appropriate conditions of storage. For a dried product to be of most use, potential for storage at room temperature is necessary. The work discussed herein would at least suggest that an attempt to further elucidate survival conditions for *L. acidophilus* during and after spray-drying might prove worthwhile.

![Figure 1. Energy of activation curves for *L. acidophilus* spray dried in 25 and 40% milk solids at constant inlet air temperature.](image-url)
TABLE 2. Percent survival of Lactobacillus acidophilus spray-dried in 40% milk solids-not-fat before and after storage for 30 days at 4 C.

<table>
<thead>
<tr>
<th>Outlet air temp. (C)</th>
<th>Moisture content (%)</th>
<th>Atmosphere</th>
<th>Dry CFU/g of solids</th>
<th>After drying (%)</th>
<th>Rehydrated CFU/g of solids</th>
<th>After 30 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>5.2</td>
<td>air N₂</td>
<td>2.6 x 10⁷</td>
<td>3.71</td>
<td>8.3 x 10⁸</td>
<td>1.19</td>
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<tr>
<td>80</td>
<td>4.3</td>
<td>air N₂</td>
<td>3.6 x 10⁶</td>
<td>0.51</td>
<td>2.5 x 10⁶</td>
<td>0.36</td>
</tr>
<tr>
<td>85</td>
<td>3.5</td>
<td>air N₂</td>
<td>1.8 x 10⁴</td>
<td>0.26</td>
<td>8.0 x 10³</td>
<td>0.11</td>
</tr>
</tbody>
</table>

TABLE 3. Percent survival of Lactobacillus acidophilus spray-dried in 25% milk solids-not-fat before and after storage for 30 days.

<table>
<thead>
<tr>
<th>Outlet air temp. (C)</th>
<th>Moisture content (%)</th>
<th>Atmosphere</th>
<th>Dry CFU/g of solids</th>
<th>After drying (%)</th>
<th>Rehydrated CFU/g of solids</th>
<th>After 30 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>6.1</td>
<td>air N₂</td>
<td>9.8 x 10⁷</td>
<td>8.20</td>
<td>4.8 x 10⁷</td>
<td>4.00</td>
</tr>
<tr>
<td>80</td>
<td>5.2</td>
<td>air N₂</td>
<td>2.9 x 10⁷</td>
<td>2.42</td>
<td>1.7 x 10⁷</td>
<td>1.42</td>
</tr>
<tr>
<td>85</td>
<td>4.7</td>
<td>air N₂</td>
<td>5.9 x 10⁶</td>
<td>0.49</td>
<td>2.5 x 10⁶</td>
<td>0.21</td>
</tr>
</tbody>
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References