A Research Note

Death Kinetics of *Lactobacillus bulgaricus* in a Spray Drying Process

P. C. TEIXEIRA, M. H. CASTRO, and R. M. KIRBY*

Escola Superior de Biotecnologia  Rua, Dr. Antonio Bernardino de Almeida, 4200 Porto, Portugal

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**ABSTRACT**

Survival of *Lactobacillus bulgaricus* during spray drying was studied at various outlet air temperatures. During spray drying the logarithmic survival ratio decreased with increased outlet air temperature with first-order kinetics; the pseudo-z value for *Lactobacillus bulgaricus* was 17.3°C.

Plots of the death-rate constant for *Lactobacillus bulgaricus* versus reciprocal outlet temperature during spray drying in skim milk show a curve with two different activation energies ($E_a$). The calculated $E_a$ values were 33.47 kJ/mol above 70°C and 85.77 kJ/mol below 70°C.

Thermodynamic quantities for spray drying of *Lactobacillus bulgaricus* are also presented. Results show that the relationship between the entropy of activation and the enthalpy of activation for both spray drying and heating in liquid medium is linear, with all the data for drying falling in the range of a negative entropy.

Key words: Spray drying, *Lactobacillus bulgaricus*, kinetics, activation energy

The development of concentrated cultures for inoculating bulk starters or the production vat directly has eliminated many of the problems customarily involved in preparing and maintaining starter cultures in the dairy plant. Since the culture concentrates can be evaluated and standardized for activity before shipment to the processor, it should be possible to produce consistently high-quality dairy products (8). Dried preparations have the advantages of long-term preservation and convenience in handling, storage, marketing, and consumption. Tamine (17) reported that freeze drying is the most commonly used technique for preparation of commercial dried starter cultures.

Freeze drying, however, is not an ideal technique. Cultures often have extended lag phases and the technique is more expensive than spray drying (16). The delay to onset of growth is largely due to a decrease in total viability but to reversible cellular damage. Injured cells must repair damage before growth commences; this means that injured cells will take longer to start their desirable activities in food fermentations (2). Teixeira et al. (19) reported that no significant differences in survival and acid production were found when *L. bulgaricus* cells were dried by spray drying or freeze drying.

The extent of survival or destruction of bacteria during spray drying may depend upon the temperature-time combinations used and upon the heat resistance of the organism. Many studies have been devoted to the influence of the air temperature on the survival of microorganisms during spray drying (6, 10, 12, 15). Lactic acid bacteria, which are frequently used as starter cultures in the food industry, have however received little attention (1, 9).

No agreement was found in the literature about the effects of operating conditions on survival of different microorganisms during spray drying. It is possible that survival might be dependent on the microbial strain or even on the type of spray dryer used.

Our aim was to study the destruction of *Lactobacillus bulgaricus* during spray drying in skim milk and to try to relate death with thermodynamic parameters. Generally *L. bulgaricus* is not used alone as starter culture. It was however selected for this study due to its exceptional sensitivity to drying processes (17).

**MATERIALS AND METHODS**

**Organism and growth conditions**

*Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 1489 was used. Cultures were maintained as in Teixeira et al. (18). De Man, Rogosa, Sharpe (MRS) broth (LAB M, Bury, UK) was inoculated from MRS agar (LAB M) slopes and incubated for 24 h at 42°C. This broth was then used to inoculate a second MRS broth (1% vol/vol). The cultures were incubated at 42°C for 24 h in a shaken water bath. Cells were harvested by centrifugation at 16,266 x g for 10 min at 4°C and washed with sterile phosphate buffer (0.01 mol/l of K$_2$HPO$_4$ [Merck, Frankfurt, Germany] and 0.01 mol/l of KH$_2$PO$_4$ [Merck], both dissolved in a solution of 0.15 mol/l of NaCl [Merck], adjusted to pH 7.0 ± 0.1 and sterilized).

**Spray drying**

Skim milk was inoculated with *L. bulgaricus* cultures. This suspension was incubated for 30 min at 37°C to allow for cell adaptation, constantly agitated, and then spray dried in a laboratory-scale spray drier (Niro Atomizer, Gladtsaxevej, Denmark). Moisture in spray droplets produced by the atomization of the feed liquid into
a spray by the use of a vaned wheel rotating at high speed was evaporated in a vertical, cocurrent drying chamber (0.8-m diameter and 0.6-m height). When studying the effect of various outlet temperatures (62°C to 105°C), all the conditions except feed rates were fixed (inlet air temperature 200°C, atomizing air pressure 5 bar). Powder was collected in a single-cyclone separator.

**RESULTS AND DISCUSSION**

Figure 1 shows that during spray drying, the logarithmic survival ratio decreased with increased outlet air temperature in a linear fashion. This is in agreement with results of Kim and Bhowmik (10) and Laubuz et al. (11) but contradicts results previously obtained by Peri and De Cesari (15). In thermobiology the influence of the temperature in heating processes on the destruction of bacteria and enzymes is expressed by the parameter z. In accordance with the recommendations of Kim and Bhowmik (10) for studies of processes which involve both heating and drying, a corresponding parameter nominated the pseudo-z value is used. The pseudo-z value shows the temperature increase needed for obtaining a 10-fold increase in destruction. Data from Figure 1 was used to calculate the pseudo-z value for *L. bulgaricus* which is 17.3°C. Kim and Bhowmik (10) reported pseudo-z values of 12°C for *Streptococcus thermophilus* and 10.6°C for *L. bulgaricus*. The higher pseudo-z value presented here may be explained by the differences in acidity between milk, used in this study, and yoghurt, employed in the earlier work.

The Ea of the death process was calculated according to Elizondo and Labuza (6) (Figure 2). The method used here to treat the results has been criticized by some authors (4) however, even if it only roughly approximates the actual complexity of the drying process, it has proved to be a useful analytical tool for the comparison of experimental data (10, 15).

Plots of the death rate constant for *L. bulgaricus* versus reciprocal outlet temperature during spray drying in skim milk show a curve with two different activation energies. This break in the curve is consistent with data obtained by other workers for other organisms (6, 7, 14, 15). The significance of breaks in the Arrhenius plot leading to abrupt changes in Ea has been the source of much controversy. Drost-Hansen (5) has shown that many of the breaks can be attributed to the properties of water itself. Elizondo and Labuza (6) pointed out the possibility that at the higher temperature the drying time is somewhat shortened. This would raise the curve and might even straighten it out. Unfortunately, no models are available to accurately calculate the exact drying time during spray drying. Peri (14) and Peri and De Cesari (15) showed that the change in the Ea takes place when residual humidity drops to values corresponding to the Brunauen Emmet and Taller monolayer. At these values of residual humidity a sharp decrease of both the rate of drying and of microorganism destruction takes place.

The calculated Ea values were 33.47 kJ/mol above 70°C and 85.77 kJ/mol below 70°C. This reduction in the Ea would suggest a different mechanism for death at higher temperatures. When comparing these values with those obtained for destruction in liquid medium (data not shown), it is observed that the drying process affects the Ea value. This was explained by Elizondo and Labuza (6) using thermodynamic parameters.

**Figure 2. Death rate constant (k) for Lactobacillus bulgaricus versus reciprocal outlet temperature during spray drying in skim milk. Vertical bars represent overall standard errors of the mean.**
This linear correlation is known as the "compensation law." This compensation law points out a thermodynamic rationale for the large drop in activation energy for death during drying. "Although the lower activation energy means that less energy is needed for the death of the microbes, the negative entropy lowers the probability of death occurring so, thus, the organisms are less heat sensitive (6)."

Working with Salmonella species, LiCari and Potter (12) found no close relationship between thermoresistance and behavior during spray drying and ascribed this to the fact that the destructive effect of spray drying rests on a combination of wet heat, dry heat, and nonthermal drying effects. It is therefore concluded that one cannot extrapolate heating death in solution to predict data during drying.

REFERENCES

Table 1. The thermodynamic parameters of death of Lactobacillus bulgaricus during spray drying in skim milk

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Reaction rate constant, k (s⁻¹)</th>
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<th>ΔH (kJ/mol)</th>
<th>ΔS (kJ/molK)</th>
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