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Inactivation of *Lactococcus lactis* ssp. *cremoris* cells in a droplet during convective drying

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ABSTRACT

Spray drying is a less costly alternative to freeze drying in the mass-production of active dry microorganisms, if the drying conditions could be optimized to preserve cell viability. As spray drying is akin to a black-box process, in this study we used an alternative approach of a single droplet drying to study how drying conditions affect the inactivation of bacterial cells. The inactivation histories of *Lactococcus lactis* ssp. *cremoris* were investigated at air temperatures of 70, 90, and 110 °C. It was found that the viability of *L. cremoris* cells could be maintained at approximately the original level for extended drying durations (60–210 s), despite the high air temperatures. When plotted against droplet temperature T_d, the inactivation rate k_d at six drying conditions formed a general trend. An inactivation model was proposed to describe different inactivation histories under varied drying conditions. The description closely followed the experimental data, reported for the first time in literature. k_d increased rapidly after T_d passed a transition temperature range of 50–65 °C, coinciding with the onset temperature for denaturation of bacterial ribosomes. Other environmental parameters affecting inactivation are discussed to better understand the integrated effects of multiple stresses experienced by bacterial cells during convective drying.

1. Introduction

Active dry microorganisms are important food ingredients for a range of products with high trading values [1,2]. These dry cultures have prolonged shelf-life, more stable properties, and are easier to transport compared to liquid cultures, while ideally still retaining similar bioactivity. Examples of commercially available dry cultures include: dry starter cultures for the dairy industry, mainly from lactic acid bacteria [3]; dry probiotics in health care and pharmaceutical products [4]; dry yeasts for fermentations in breweries and bakers' industries [5]; and bio-preservatives inhibiting the growth of other microorganisms to replace antibiotics in feed products [6]. Traditionally, dry cultures are produced by freeze drying, which requires relatively long processing times and high energy consumption [7,8]. Recently, spray drying as a conventional dehydration approach to process heat-sensitive materials into powders has attracted interest for the production of active dry microbial cultures [1,9]. In reported studies, bacteria and yeast could survive a spray drying process with high survival ratios of up to 80–100%, which compares favorably with the freeze drying process [10–13].

During spray drying, a feed solution containing microorganisms and often a carrier material to protect these microorganisms is first atomized into droplets and then sprayed into a drying tower. Hot air flows, often more than 200 °C, are used in the drying tower to quickly remove the moisture from the droplets. Evaporation occurs rapidly as a result of the high air temperature and the large contact area, producing dried particles of the carrier where the microorganisms are encapsulated. During the simultaneous heating and dehydration processes, microbial cells would suffer from multiple stresses such as thermal [14], osmotic [15], and oxidative stresses [16], which can lead to irreversible cell death. Fu and Chen [1] discussed nine extrinsic parameters affecting the survival of microbial cells during convective drying. Briefly, they can be grouped as (i) drying air properties (air temperature and humidity); (ii) kinetic drying parameters (material temperature T_d, initial moisture content X_0, moisture removal rate $-dX/dt$, temperature variation rate dT/dt and exposure time t); and (iii) carrier properties (carrier composition and the location of cells inside the carrier). A reliable mathematical model correlating the inactivation of microbial cells to these parameters would not only optimize individual spray drying operations, but also contribute to understandings of

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Nomenclature

E_d	activation energy for deactivating cells (kJ/mol)
k_0	a pre-exponential factor
k_d	rate constant of cell deactivation
N	number of viable cells (cfu/mL)
N_0	initial number of viable cells (cfu/mL)
t	time of drying (s)
T_d	droplet temperature (°C or K)
X	average moisture content on dry basis (kg/kg)

the effects of each factor. Previous reported inactivation models often relate the cell death rate to the dryer outlet temperature [17–19]. However, the activation energy of the cell inactivation calculated in this manner would be inaccurate, as droplets/particles inside the dryer usually experience wide temperature and moisture variations [1,18]. Typically average values for all the droplets are considered in previous studies, which inhibits the accurate understandings of drying conditions on cell viability. The sub-cellular mechanism of spray drying to deactivate microorganisms is also poorly understood and constitutes object of further studies [20]. Droplets during spray drying would experience different drying histories due to the differences in initial sizes and droplet trajectories [21,22]. This wide thermal and moisture history variability is a challenge to interpret, making the development of inactivation models for individual droplets a very difficult task.

Single droplet drying (SDD) is an established experimental technique to study droplet drying behaviour in a controlled environment mimicking spray drying conditions [23,24]. The technique is capable of accurately measuring changes in the droplet weight, temperature and diameter as drying progresses. This kinetic data is essential for developing models [25] as well as to validate CFD simulations of spray drying processes [26–28]. SDD has been shown to produce powders with the same morphological properties to spray dryers [29–31]. For these reasons, SDD technique has hence become a powerful tool to study any droplet-drying-related phenomenon, such as those occurring during spray drying. Previously, SDD systems have been employed to study the inactivation kinetics of different bacteria [32] and effects of various carriers [33]. In the present study, the inactivation of a model microorganism under different drying conditions was monitored to investigate the correlation between cell inactivation and droplet drying histories. The model microorganism used was *Lactococcus lactis* ssp. *cremoris*, a typical Gram-Positive cocci used as a starter culture in the cheese industry. An inactivation model was developed to describe inactivation histories under varied conditions, focusing also on the effects of extrinsic parameters on cell survival.

2. Materials and methods

2.1. Microorganism and culture conditions

The culture of *Lactococcus lactis* ssp. *cremoris* was maintained on standard M17 (Oxoid CM0817) agar plate at 4 °C with subculture to fresh media every seven days. For single droplet drying (SDD) experiments, 10 mL of M17 culture medium were inoculated with the strain and then incubated in a 30 °C stationary incubator for 24 h prior to drying experiments.

2.2. Preparation of cells for drying experiments

After 24 h incubation, *L. cremoris* cells were collected by centrifugation at 10,000 rpm, 25 °C for 10 min. The resultant cell pellets were re-suspended in 10 mL carrier solution, in order for the viable cell concentration in the carrier solution to be equivalent to that of the 24 h culture. The carrier used was reconstituted skim milk (RSM), with 10 or 20 wt% solids content. Skim milk powder was purchased locally, consisting of 37.97 wt% protein, 1.34 wt% fat, 58.96 wt% sugar, and 1.73 wt% minerals according to the product specification. The powder was reconstituted in Milli-Q water (QGARD00R1 Milli-Q system, Millipore, Australia) and sterilization was effected by autoclaving at 105 °C for 10 min. The RSM carrier solution with the re-suspended cells was placed in an ice bath to prevent undesired cell proliferation during the SDD experiments. The viable cell concentration in the carrier solution was checked immediately after preparation and after 4 h of preparation, to ensure that there was no cell reproduction and that the viable cell concentration was similar to freshly prepared samples.

2.3. Single droplet drying (SDD) experiment

The working principle and experimental set-up of the SDD system used in the present study have been described elsewhere [34,35]. Briefly, an air stream with controlled temperature, velocity and humidity was used for drying of a suspended single droplet in a confined chamber. During drying, the single droplet was suspended at the tip of a specially-made fine glass filament; while changes in droplet temperature and mass were measured using different droplet suspension modules in separate drying runs with identical conditions. A 5 μL GC micro-syringe was used to generate single RSM droplets containing *L. cremoris* cells for each run. The initial size of each droplet was 2 ± 0.05 μL, where the error was taken as half of the minimal graduation of the micro-syringe. After each drying run, the syringe was rinsed three times, with Milli-Q water, 70% (v/v) ethanol, and sterilized Milli-Q water, respectively, to avoid cross-contamination. Drying conditions used in the present study are summarized in Table 1. Under these drying conditions, Reynolds number was around 55–65 and the Nusselt number was around 6.2–6.6 at the beginning of the drying experiment. The value of both dimensionless numbers decreased as drying progressed, approaching 22–28 and 4.7–5.0, respectively, at the final stage of drying.

For each drying condition, droplet mass, temperature and *L. cremoris* viability were monitored as drying progressed. Each experiment was repeatedly carried out twice, and results reported here are the averages of duplicate experiments. The droplet weight and temperature were recorded following a previously described procedure [34,35]. Viability measurements were performed on repeated SDD runs under identical drying conditions, but stopped at different times: at 0, 15, 30, 45, 60, 90, 180, 210, 240, 270, and 300 s. The resulting semi-dried particle, with an initial volume of 2 μL, was dissolved in situ with 2 mL of M17 diluents without removing it from the suspending glass filament. The residual viable cells in each semi-dried particle were estimated using the standard plate count procedure, where M17 diluents were used for serial dilutions. The M17 diluents were 1/10 of the strength of the original M17 medium without lactose. As such, each viable cell data corresponded to a separate SDD process. Duplicate experiments of viability measurement were independently carried out using a fresh 24 h culture.

2.4. Visualisation of dried bacterial cells

SEM imaging was conducted for RSM particles with an initial solids content of 10 wt%. These particles were dried for 6 min at 70 °C, 5 min at 90 °C, and 4 min at 110 °C, to achieve a relatively complete water removal. At the end of each drying run, the formed particles were removed from the suspending glass filament and carefully divided into smaller pieces for SEM imaging. At least two specimens were used for each drying temperature. Samples were