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Agent selection and protective effects during single droplet drying of bacteria

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ABSTRACT

The protective mechanisms of whey protein isolate (WPI), trehalose, lactose, and skim milk on *Lactobacillus plantarum* A17 during convective droplet drying has been explored. A single droplet drying technique was used to monitor cell survival, droplet temperature and corresponding changes in mass. WPI and skim milk provided the highest protection amongst the materials tested. *In situ* analysis of the intermediate stage of drying revealed that for WPI and skim milk, crust formation reduces the rate of sudden temperature increase thereby imparting less stress on the cells. Irreversible denaturation of the WPI components might have also contributed to the protection of the cells. Skim milk, however, 'loses' the protective behaviour towards the latter stages of drying. This indicates that the concentration of the WPI components could be another possible factor determining the sustained protective behaviour during the later stages of drying when the moisture content is low.

1. Introduction

Lactic acid bacteria (LAB) are a group of ubiquitous Gram positive bacteria which are generally regarded as safe (GRAS) in food preservation. LAB, especially those in the genus *Lactobacillus*, are most widely utilised in food and theopathic applications (Bourdichon et al., 2012; Vogel et al., 2011). In addition, LAB can enhance food safety and consumer health by preventing or reducing the incidence of pathogens (Gaggìa, Gioia, Baffoni, & Biavati, 2011; Gaggìa, Mattarelli, & Biavati, 2010).

The success in the utilisation of the LAB cells for such purposes often requires high cell density and retention of activity for a reasonable amount of time before incorporation into the food formulation, in order to ensure the desired effect. A variety of different methods have been reported in the literature, including freeze drying, spray drying, vacuum drying, air drying and fluidised bed drying (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008) and these have resulted in varying degrees of cell survival.

Amongst the processing techniques for producing dried microbial cells, optimising spray drying and developing product formulations to minimise activity losses have attracted increasing research interest as it is much more cost effective than freeze drying and larger volumes can be processed (Knorr, 1998). Spray drying involves the fine atomisation of a liquid feed material and the subsequent rapid evaporation of water makes it especially suitable for heat sensitive products. Yet, the success in utilising this approach has been based on trial and error, while different research groups have reported very variable rates of cell survival ranging from less than 1–100% (Fu & Chen, 2011). Therefore, the objective of enhancement in cell viability warrants further investigation and an insight into the inactivation mechanisms of cells is a prerequisite (Santivarangkna, Kulozik, & Foerst, 2008).

Several factors have been reported to affect cell survival during spray drying, including the tolerance of the cells as well as processing parameters applied during the drying stages (Meng et al., 2008). It has been reported that the inactivation of microorganisms may occur due to high temperature and dehydration stress (Fu & Chen, 2011; Meng et al., 2008; Peighambardoust, Golshan, & Hesari, 2011). Damage to macromolecules particularly including DNA and RNA, as well as cell membranes and ribosomes results from the exposure of the cell to high temperature. In addition, during dehydration, damage to cytoplasmic membranes can occur, leading to the loss of some intracellular components (Ananta, Volkert, & Knorr, 2005).

In order to overcome the stress encountered during spray drying, microbial cells are often formulated with various protectants, and research efforts have been devoted to the choice of agents providing optimal survival (Leslie, Israeli, Lighthart, Crowe, & Crowe,

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1995). Skim milk and carbohydrates in the form of disaccharides used either alone, or in combination, have been the most commonly used protective substances in dehydration of microorganisms in the last decade with varying effects upon cell survival. Various hypotheses have been proposed to explain the beneficial effects of sugars including the involvement of water replacement and vitrification (Santivarangkna, Higl, & Foerst, 2008). Although the protective mechanism of skim milk has not been fully explored, it has been suggested that lactose in skim milk interacts with the cell membrane and helps to maintain membrane integrity in a manner similar to the protection by other sugars including trehalose (Corcoran, Ross, Fitzgerald, & Stanton, 2004). Another major constituent of skim milk is protein and whether or not this exerts a significant protective effect remains to be elucidated (Fu & Chen, 2011).

There has been an increasing number of reports on the application of milk protein in the protection of probiotic cells in recent years, relating to roles in protection both whilst drying is occurring as well as during exposure to gastrointestinal or bile fluids. This probably reflects the desirable gelation properties of the proteins which have been shown to be useful for the microencapsulation of probiotics (Rathore, Desai, Liew, Chan, & Heng, 2013). There have been a few reports specifically on the application of whey for the protection of probiotics during spray drying. These have demonstrated good survival; however, these studies utilised either whey protein isolates (WPI) in combination with carbohydrate or liquid whey which also contains lactose (De Castro-Cislaghi, Silva, Fritzen-Freire, Lorenz, & Sant'Anna, 2012; Ying et al., 2011, 2013). Furthermore, none of these studies has addressed the mechanism(s) whereby the probiotics were protected.

During pilot scale spray dying, billions of droplets are sprayed into a relatively large tower. As a result of the varied trajectories the droplets could have quite different drying histories, posing challenges to understand bacterial inactivation and the mechanisms of protection afforded by various drying media. To circumvent these difficulties, a single droplet drying device mimicking the spray drying environment was employed here to investigate different inactivation histories of bacterial cells as the droplet was being dried under controlled conditions. This single droplet drying device allows accurate measurements of changes in the droplet temperature, mass and diameter as drying progresses (Fu, Woo, Lin, Zhou, & Chen, 2011). Therefore, the objective of this study was to examine the protection mechanism of *Lactobacillus plantarum* A17 during convective drying using WPI, lactose, skim milk, mixtures of lactose and WPI and the well-known non-reducing disaccharide trehalose.

2. Materials and methods

2.1. Materials

2.1.1. Protectants

Five forms of protectant were used: (1) WPI 894 (Fonterra, Australia) in both pasteurised and native forms; (2) trehalose dihydrate (T9531, Sigma Aldrich, Australia); (3) lactose monohydrate (L3625, Sigma Aldrich, Australia); (4) long life skim milk (purchased from a local supermarket and used without further treatment) and (5) a mixture of lactose and WPI in a ratio of 9.94:0.06. Deionised water (Milli-Q system QGARD00R1, Millipore, Australia) was used in all experiments.

2.1.2. Lactobacillus plantarum A17

The test strain of *L. plantarum* A17 was obtained from the collection of the Laboratory of Food Microbiology, School of Applied Sciences, RMIT University, Australia. The strain was frozen at −80 °C in MRS Broth (Oxoid, Australia) with 40% (v/v) glycerol. Bacteria cells were sub-cultured and grown to the stage of peak log-phase in deMann, Rogosa and Sharpe (MRS) broth.

2.1.3. deMann Rogosa Sharpe (MRS) agar

This was obtained from Oxoid, Australia and contained peptone (1%), meat extract (0.8%), yeast extract (0.4%), glucose (2%), sodium acetate trihydrate (0.5%), polysorbate 80 (0.1%), dipotassium hydrogen phosphate (0.2%), triammonium citrate (0.2%), magnesium sulphate heptahydrate (0.02%), manganese sulphate tetrahydrate (0.005%) and agar (1.0%). MRS agar was used for further growth and identification of LAB colonies.

2.2. Sample preparation and measurements

2.2.1. Preparation of protectants

Protectant solutions were prepared by mixing the selected components at a concentration of 10% (w/w) in deionised water without taking into consideration the inherent moisture content in the original powder. WPI solution was dispersed by mixing with magnetic stirring for at least 30 min, stored at 4 °C overnight to ensure full hydration and the pH of the resulting solution was 6.6. Lactose and trehalose solutions were autoclaved at 121 °C for 15 min before use. For the WPI solution, two solutions were used: a solution pasteurised to 70 °C for 1 min and a non-pasteurised solution (native). As WPI tends to denature at temperatures beyond 65 °C, the latter solution was used as a control having minimal initial denaturation. Sterile deionised water (without protectant) was also used as a control.

2.2.2. Micro differential scanning calorimetry

To determine the extent of whey protein denaturation during pasteurisation, micro differential scanning calorimetry, Setaram Micro DSC VII (Setu-rau, Caluire, France) was used. Approximately 800 mg sample was filled into a DSC pan and sealed hermetically; a reference pan was also taken with deionised water of equal weight. Both pans were placed into the instrument chamber and equilibrated for 1 h at 20 °C to eliminate the effect of thermal history prior to heating to 95 °C at a programmed heating rate of 1 °C/min. Duplicate measurements were taken. During the micro differential scanning calorimetry analysis, the remaining undenatured protein in the pasteurised sample undergoes denaturation, and thus the enthalpy of denaturation is a measure of the amount of undenatured protein present in the sample after pasteurisation. The enthalpy values were calculated from the area under a line extending from 60.2 to 87.5 °C and the values were normalised to give enthalpies per gram of dry protein.

2.2.3. Preparation of cells for drying experiments

L. plantarum cells were grown in MRS broth for 17 h to reach the late growth phase. The active growing cells were homogeneously mixed and harvested by pipetting 1 ml into each of a number of Eppendorf tubes before centrifugation at 4000*g* for 10 min. After decanting the supernatant, the pellet of cells in each tube was washed with sterile saline water (0.85%) and then resuspended in one of a series of different protectant dispersions (10% w/w) with a tube containing deionised water as a control. The tubes with the cell suspensions in carrier media and water were all stored at room temperature (~24 °C) for approximately 30 min before commencement of the drying experiments. All tubes were placed in an ice bath throughout the drying experiments to prevent undesired cell proliferation. The viable cell concentration in the samples was checked immediately after preparation as well as after 4 h to ensure that there was no cell proliferation. All suspensions were mixed by gently vortexing before the withdrawal of sub-samples for drying. Initial cell concentrations were 2–3 $\times$ 10^9 CFU ml^{-1}.