Food Research International 76 (2015) 478–488

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

The mechanisms of the protective effects of reconstituted skim milk during convective droplet drying of lactic acid bacteria

Xufeng Zheng [a], Nan Fu [a,*], Manlei Duan [a], Meng Wai Woo [b], Cordelia Selomulya [b], Xiao Dong Chen [a,*]

[a] Suzhou Key Laboratory of Green Chemical Engineering, School of Chemical and Environmental Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou City, Jiangsu 215123, P.R. China
[b] Department of Chemical Engineering, Monash University, Clayton Campus, Wellington Rd., Victoria 3800, Australia

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 18 July 2015
Accepted 24 July 2015
Available online 1 August 2015

Keywords:
Sing droplet drying
Spray drying
Inactivation kinetics
Calcium effect
Lactobacillus rhamnosus GG
Lactococcus lactis ssp. *cremoris*

ABSTRACT

Reconstituted skim milk (RSM) is a reputed protective carrier for improving the survival ratio of lactic acid bacteria (LAB) after spray drying; however the underlying mechanisms of the prominent protection remains unclear. In this study, the inactivation histories of two LAB strains during droplet drying with four carriers were experimentally determined, and the effects of droplet drying parameters on LAB inactivation were investigated. For the first time, the possible contribution of each RSM components to the maintenance of LAB viability during drying was discussed. Rapid inactivation of LAB cells only started at the later stage of drying, where RSM could maintain viability better upon both high droplet temperature and low moisture content than the other three carriers tested. Such protective effects was attributed to calcium and milk proteins rather than lactose. Upon the rapidly increasing droplet temperature at the later stage, calcium might enhance the heat resistance of LAB cells, whereas proteins might lead to a mild temperature variation rate which was beneficial to cell survival. LAB cells dried in the reconstituted whole milk showed the most advanced transition of rapid viability loss, with transition temperature at around 60 °C, in contrast to 65-70 °C in lactose and MRS carriers and 75 °C in the RSM carrier. The detrimental effects could be due to the high level of milk fat content. The proposed effects of each RSM components on LAB viability would be useful for constructing more powerful protectants for production of active dry LAB cells via spray drying.

1. Introduction

Dried lactic acid bacteria (LAB) with retained activity have wide applications in food industries. They can be used as probiotics that confer health benefits to host through oral administration (Masood, Qadir, Shirazi, & Khan, 2011), as fermentation starter culture for cheese and yogurt production (Dijkstra et al., 2014), and as bio-preservatives for adding in animal feeds to inhibit the growth of pathogens (Schnürer & Magnusson, 2005). The dry powder form compared to liquid culture facilitates the subsequent processing procedures such as pelletization or tabletting, as well as transportation (Fu & Chen, 2011; Peighambardoust, Golshan Tafti, & Hesari, 2011). Traditionally, microorganisms are preserved through freeze drying, which provides the maximal viability, but is time- and energy-consuming. In recent years, producing active dry microorganisms via spray drying has attracted increasing research attention owing to several advantages, e.g., fast dehydration with high production capacity, excellent powder properties, one-step production of microcapsules, and capability of processing heat-sensitive materials (Rajam & Anandharamakrishnan, 2015; Schuck, Dolivet, Méjean, Hervé, & Jeantet, 2013). A major limitation of spray drying stems from the trial-and-error processes needed to select the optimal operating conditions (Haque, Aldred, Chen, Barrow, & Adhikari, 2014; Schutyser, Perdana, & Boom, 2012), in order to achieve the highest cell survival ratio after drying. Under nonoptimal conditions, bacterial cells could suffer multiple environmental stresses due to the high air temperature (Fu, Woo, Selomulya, & Chen, 2013), fast changes in osmotic pressure (Perdana et al., 2013), and the associated oxidative stress (Garre, Raginel, Palacios, Julien, & Matallana, 2010). Each stress could cause damages to multiple cellular structures including cytoplasmic membrane, ribosomes, and genome DNA (Khem, Woo, Small, Chen, & May, 2015).

Previous studies often adopted two approaches to increase cell survival ratio after spray drying, i.e., the adjustment of dryer settings (Ghandi, Powell, Broome, & Adhikari, 2013; Sunny-Roberts & Knorr, 2009; Wang, Yu, & Chou, 2004) and the attempt of different feed solutions to carry bacterial cells (Lian, Hsiao, & Chou, 2002; Rajam & Anandharamakrishnan, 2015; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014). Similar to the cyto- and osmotic-protectants used in freeze drying, certain chemicals show prominent protective effects towards the viability of dried LAB cells, amongst which a reputed one is reconstituted skim milk (RSM). RSM carrier could retain higher viability of spray dried LAB than a number of other carriers tested, including gelatin, maltodextrin, polydextrose

* Corresponding authors.
E-mail addresses: nan.fu@suda.edu.cn (N. Fu), xdchen@suda.edu.cn (X.D. Chen).

http://dx.doi.org/10.1016/j.foodres.2015.07.045
0963-9969/

and whey permeate (Corcoran, Ross, Fitzgerald, & Stanton, 2004; Lian et al., 2002; Reddy, Madhu, & Prapulla, 2009; Riveros, Ferrer, & Borquez, 2009). However, mechanisms underlying the outstanding protective effects of RSM are still under debate. Early study tended to attribute the protection of RSM to lactose, a disaccharide, which was assumed to protect cytoplasmic membrane during dehydration with similar mechanisms to those non-reducing disaccharides such as trehalose and sucrose (Ananta, Volkert, & Knorr, 2005). However, lactose per se did not demonstrate much protective effects (Perdana, Fox, Siwei, Boom, & Schutyser, 2014; Riveros et al., 2009). In recent studies, protection from milk protein components has drawn much attention. When skim milk was denatured by either heat or heat plus calcium cation (Ca^{2+}), the denatured milk proteins were found to improve the resistance of LAB strains against heat stress (Huang et al., 2014). Whey protein component has been suggested as the main cause for the protective effects of RSM (Khem et al., 2015), but there remains controversy in the literature. Some spray drying studies using whey protein as carrier lacked the contrast results from RSM (Duongthingoc, George, Katopo, Gorczyca, & Kasapis, 2013; Ying et al., 2013), whereas others used whey protein as the secondary encapsulating materials in addition to maltodextrin (Soukoulis et al., 2014). Moreover, Mañas, Pagán, Sala, and Condón (2001) found that demineralized whey components lost the protective effects on bacterial cells against heat, but could regain the effects when divalent cations such as calcium and magnesium were added.

As a complex medium containing lactose, fat, casein, whey protein, and abundant inorganic salts, a detailed analysis of the mechanisms underlying the protection of RSM would be helpful for constructing more powerful protectants for spray drying operation. The present study investigated the performance of RSM on protecting the viability of two LAB strains during convective droplet drying, in contrast to three other media, i.e., lactose solution, growth medium supplement with lactose, and reconstituted whole milk (RWM). Drying experiments were carried out with a single droplet drying (SDD) device mimicking spray drying conditions, which enabled cell inactivation histories to be monitored as drying progressed. The inactivation histories of each LAB strain were analysed in relation to changes in droplet temperature and moisture content for all carrier media tested. The possible contribution of each RSM components to the observed protective effects during drying of LAB cells was discussed.

2. Materials and methods

2.1. Microorganisms and culture conditions

Lactobacillus rhamnosus GG (LGG) was kindly provided by CVS Pharmacy (Culturelle®, New Haven, CT, USA). The strain was cultured on MRS agar plate using streak plate technique. MRS agar plate was prepared by adding 12.0 g/L agar (A8190, Beijing Solarbio Science & Technology Co. Ltd, Beijing, China) to MRS broth (Oxoid CM1175, Oxoid Ltd, Hampshire, UK). *Lactococcus lactis* ssp. *cremoris* was cultured on M17 agar plate prepared by adding 12.0 g/L agar to M17 broth (Oxoid CM0817, Oxoid Ltd, Hampshire, UK). The culture plates were stored at 4 °C. Both organisms were sub-cultured on to fresh media every seven days, by transferring a single colony to a fresh agar plate using streak plate technique and then incubating the plate for 48 h. The incubation temperature of LGG was 37 °C and that of *L. cremoris* was 30 °C.

Before SDD experiments of LGG, 10 mL of sterilized MRS medium was inoculated with a single colony from the culture plate and then incubated at 37 °C for 24 h. The culture of *L. cremoris* cells for SDD experiments followed a similar procedure, except that 10 mL of M17 medium was used and the incubation temperature was 30 °C.

2.2. Materials

Lactose was purchased from Sigma Aldrich (L8783, Sigma-Aldrich Shanghai Trading Co. Ltd, Shanghai, China). Skim milk (SMP) and whole milk powders (WMP) were purchased locally (Anchor®, Fonterra Brands (NZ) Ltd, Auckland, New Zealand). The composition of the SMP was 33 g protein, 1 g fat, 54 g carbohydrate, 0.39 g Sodium and 1.2 g Calcium per 100 g solids, whereas that of the WMP was 24 g protein, 28.8 g fat, 38.4 g carbohydrate, 0.28 g Sodium, and 0.96 g Calcium per 100 g solids, according to product specification.

For SDD experiments, 10 wt.% solution of each material was prepared using Milli-Q water (Milli-Q® Direct 16, Merck Millipore, Darmstadt, Germany). Growth medium carrier of LGG was prepared by dissolving 0.52 g MRS broth powder (Oxoid CM1175) and 0.59 g lactose powder in 10.0 g water to construct 10 wt.% solution, whereas that of *L. cremoris* was prepared by dissolving 0.37 g M17 broth powder (Oxoid CM0817) and 0.63 g lactose powder in 9.0 g water. The different preparation methods were to control the fraction of lactose in the final solution. All carrier solutions were autoclaved at 105 °C for 10 min and cooled to room temperature before the addition of LAB cells.

2.3. Preparation of cell suspensions for drying experiments

Feed solutions containing bacterial cells for drying experiments were prepared following a modified procedure as previously reported (Fu et al., 2013). A schematic overview of the preparation procedure is presented in Fig. 1. Firstly, 1 mL of the LAB culture after 24 h incubation was centrifuged at 8,000 rpm, 4 °C, for 15 min (Neofuge 13R, Heal Force Bio-meditech Holdings Ltd, Shanghai, China) to collect cell pellet. Then the pellet was washed twice with 0.5% (w/v) peptone solution (P8450, Beijing Solarbio Science & Technology Co. Ltd, Beijing, China) and re-centrifuged, followed by re-suspension in 1 mL carrier solution. As such the feed for SDD experiments contained the same concentration of viable cells as the 24 h culture. Carrier solutions with LAB cells were placed in ice bath after preparation, to ensure that the viable cell amount stayed similar as freshly prepared during SDD experiments.

2.4. Single droplet drying experiment

The single droplet drying rig (SDPA MARK II, Dong-Concept New Material Technology Co. Ltd, China) and working principles have been described previously (Fu et al., 2012; Tian et al., 2014). Briefly, the rig consisted of two systems: an air supply system to condition compressed air into laminar flow with controllable temperature, velocity and humidity, and an droplet drying system where an isolated droplet could be suspended at the tip of a specially made glass filament for drying in the conditioned air stream. The droplet drying system was equipped with three droplet suspension modules. Each module contained a suspension glass filament specially designed for the measurement of a droplet parameter while drying was in progress. The changes in droplet mass, temperature and diameter during drying can be measured using the corresponding suspension module in separate drying runs with identical conditions. The procedures of measurement of each droplet parameter have been described in details in previous publications (Fu, Woo, Lin, Zhou, & Chen, 2011; Fu et al., 2012).

The single droplets for drying experiments were generated using a gas chromatograph micro-syringe (Part#001100, SGE Analytical Science Pty Ltd, Australia). The initial size of each droplet was 2 ± 0.05 μL; the error was taken as half of the minimal graduation of the micro-syringe. After each drying run, the syringe was rinsed by Milli-Q water, 70% (v/v) ethanol, and sterilized Milli-Q water subsequently, to eliminate cross-contamination of bacteria between drying runs. Drying conditions used in the present study are shown in Table 1. A schematic overview of the experimental procedures of SDD measurements is shown in Fig. 1.