J. Dairy Sci. 96:1419–1430
http://dx.doi.org/10.3168/jds.2012-6058


# Effect of drying methods of microencapsulated *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. *cremoris* on secondary protein structure and glass transition temperature as studied by Fourier transform infrared and differential scanning calorimetry

**Dianawati Dianawati,* Vijay Mishra,† and Nagendra P. Shah‡[1]**
*Tribhuwana Tunggadewi University, Jalan Telaga Warna, Malang 65145, East Java, Indonesia
†School of Biomedical and Health Sciences, Victoria University, Werribee campus, PO Box 14428, Melbourne, Victoria 8001, Australia
‡Food and Nutrition Science, School of Biological Science, The University of Hong Kong, Pokfulam Road, Hong Kong

## ABSTRACT

Protective mechanisms of casein-based microcapsules containing mannitol on *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. *cremoris*, changes in their secondary protein structures, and glass transition of the microcapsules were studied after spray- or freeze-drying and after 10 wk of storage in aluminum foil pouches containing different desiccants (NaOH, LiCl, or silica gel) at 25°C. An in situ Fourier transform infrared analysis was carried out to recognize any changes in fatty acids (FA) of bacterial cell envelopes, interaction between polar site of cell envelopes and microcapsules, and alteration of their secondary protein structures. Differential scanning calorimetry was used to determine glass transition of microcapsules based on glass transition temperature ($T_g$) values. Hierarchical cluster analysis based on functional groups of cell envelopes and secondary protein structures was also carried out to classify the microencapsulated bacteria due to the effects of spray- or freeze-drying and storage for 10 wk. The results showed that drying process did not affect FA and secondary protein structures of bacteria; however, those structures were affected during storage depending upon the type of desiccant used. Interaction between exterior of bacterial cell envelopes and microencapsulant occurred after spray- or freeze-drying; however, these structures were maintained after storage in foil pouch containing sodium hydroxide. Method of drying and type of desiccants influenced the level of similarities of microencapsulated bacteria. Desiccants and method of drying affected glass transition, yet no $T_g \leq 25$°C was detected. This study demonstrated that the changes in FA and secondary structures of the microencapsulated bacteria still occurred during storage at $T_g$ above room temperature, indicating that the glassy state did not completely prevent chemical activities.

**Key words:** desiccant, glass transition temperature ($T_g$), cell envelope, secondary protein

## INTRODUCTION

The use of particular drying methods to preserve probiotic bacteria provides some advantages besides its ease of handling, including low cost of transportation and storage at room temperature. Freeze-drying and spray-drying are 2 common drying methods for preservation of bacteria; however, these have many adverse effects on cell envelopes and secondary protein structures (Leslie et al., 1995; Mauerer, 2006). Microencapsulation technology has been developed to overcome these problems. The application of sodium caseinate-glucose to form a glassy Maillard substance, combined with mannitol, is effective in protecting spray-dried probiotic bacteria (Crittenden et al., 2006). Mannitol is excellent in protecting probiotic bacteria during storage and exposure to a simulated gastric environment due to its radical scavenging ability and structural stability in low pH (Efiuvwevwere et al., 1999; Telang et al., 2003), in spite of its tendency to crystallize (Izutsu and Kojima, 2002).

Mechanisms of dehydrated bacterial protection by sugars can be explained by water replacement theory (Crowe et al., 1988) or the formation of amorphous state (Santivarangkna et al., 2011). The Fourier transform infrared (**FTIR**) technique has been used to investigate the role of sugars in retarding conformational changes of bacterial cell envelopes and proteins (Leslie et al., 1995; Oldenhof et al., 2005; Santivarangkna et al., 2010). The wave number alteration indicated that the protective mechanism of cell envelopes of bacteria occurs through sugar interaction with phospholipid headgroups via hydrogen bond (Crowe et al., 1988; Grdadolnik and Hadzi, 1998). Gauger et al. (2002)

Received August 14, 2012.
Accepted December 4, 2012.
[1]Corresponding author: npshah@hku.hk

stated that certain levels of water activity ($\mathbf{a_w}$) at room temperature contributed to conformational disorder of diphytanoylphosphatidylcholine. Protein conformation was also affected by freeze- and spray-drying (Garzon-Rodriguez et al., 2004; Schüle et al., 2007); drying process and storage at room temperature at low $a_w$ might cause the changes in cell envelopes and secondary structure of proteins of bacteria.

Apart from molecular interaction between cell envelopes and microencapsulants, the physical state of microcapsule matrix is also crucial for bacterial stability. The extremely high viscosity of dehydrated products in the amorphous state is capable of decreasing molecular mobility reducing adverse chemical reactions; however, this solid state is metastable and strongly depends on the glass transition temperature ($\mathbf{T_g}$). Storage at room temperature above $T_g$ might increase the chance of glass transition (Santivarangkna et al., 2011), in which molecular mobility would increase along with the formation of crystalline state. Glass transition temperature is also influenced by $a_w$ of storage: an increase in $a_w$ results in a decrease in $T_g$ (Higl et al., 2007; Kurtmann et al., 2009). The mechanism of bacterial protection by sugars during dehydration has been established, but the effect of long-term storage at room temperature on the changes in phospholipid bilayers and secondary protein structures of bacterial cells has not. The aims of this study were to ascertain the interaction between cell envelopes of bacteria and encapsulant, as well as to determine the changes in the structure of secondary proteins and to establish $T_g$ and moisture content of microcapsules after spray- or freeze-drying and after 10 wk of storage in aluminum foil pouches containing different desiccants at 25°C. One probiotic bacteria (*Lactobacillus acidophilus*) and one sensitive lactic acid bacteria (*Lactococcus cremoris* ssp. *lactis*) were used as models in this study.

## MATERIALS AND METHODS

### *Lb. acidophilus 2401 and Lc. lactis ssp. cremoris R-704 and Their Cultivation*

Pure cultures of *Lactobacillus acidophilus* 2401 (*Lb. acidophilus*) and *Lactococcus lactis* ssp. *cremoris* R-704 (*Lc. cremoris*) were obtained from Victoria University stock culture and were confirmed using Gram staining (Ding and Shah, 2009). *Lactobacillus acidophilus* was grown in de Man, Rogosa, and Sharpe broth at 37°C for 18 h (Riveros et al., 2009), whereas *Lc. cremoris* was grown in M17 supplemented with 0.5% glucose at 30°C for 18 h (Kimoto et al., 2003); both organisms were subcultured 3 times. The cells were concentrated by centrifuging the broth at 14,000 × *g* for 15 min at 4°C (Vinderola and Reinheimer, 2003). The resultant cell pellet was washed twice with 0.85% of sterilized saline solution and then resuspended in the same solution (10 mL of cell pellet was added by 10 mL of saline solution). The initial population of concentrated bacteria was $3.1 \times 10^{10}$ cfu/mL for *Lb. acidophilus* and $1.1 \times 10^{10}$ cfu/mL for *Lc. cremoris.*

### *Preparation of Microcapsules*

Microencapsulation was performed using an oil-in-water emulsion system comprising vegetable oil (10% wt/vol), sodium caseinate (6% wt/vol), fructooligosaccharides from chicory (2% wt/vol), D-glucose (3% wt/vol), and mannitol (3% wt/vol). All of the materials were from Sigma Aldrich Corp. (St. Louis, MO) except vegetable oil, which was obtained from a local supermarket. The materials were mixed and homogenized using a magnetic stirrer, and were heated at 95°C for 30 min to initiate the Maillard reaction. One-fifth of the concentrated bacteria were incorporated to the cold emulsion system (10°C) before spray- or freeze-drying. The emulsion was spray-dried using a Buchi Mini spray drier (model B290, Bern, Switzerland) with Dehumidifier B296 (humidity 86%; temperature −3°C; Buchi). The outlet temperature was 50°C, hence the inlet temperature was set to 99°C with pump 27% (feeding rate = 7.14 mL/min) for the emulsion system containing *Lb. acidophilus*, and was set to 80°C with pump 20% (feeding rate = 3.03 mL/min) for the emulsion system containing *Lc. cremoris*. The powder gathered from the collection vessel was then stored in desiccators. For freeze-drying, frozen microcapsules were loaded into a freeze-drier (model FD-300, Airvac Engineering Pty. Ltd., Dandenong, Australia) set to achieve −13,332.2 Pa of internal pressure before freeze-drying at a temperature of −88°C, with 44 h of primary freeze-drying, and 4 h of secondary freeze-drying. Each of the freeze-dried and spray-dried products (*Lb. acidophilus* and *Lc. cremoris*) were placed on Petri-disks and kept in desiccators containing a saturated solution of sodium hydroxide (NaOH; $a_w = 0.07$), a saturated solution of lithium chloride (LiCl; $a_w = 0.11$), or silica gel for 2 wk to reach the equilibrium. Once equilibrium was established, the products were transferred to aluminum foil pouches, and NaOH, LiCl, or silica gel was packed inside a semi-permeable membrane and placed inside the pouch. Controls were stored without desiccant, fresh samples were freshly harvested bacteria after being grown in media for 18 h, and prestorage samples were after freeze drying/after spray drying. Storage at 25°C was carried out for 10 wk; after the end of storage period, samples were kept at −80°C until further analysis.