Shellac coating enhances survival of probiotics during storage and the possible mechanisms

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INTRODUCTION

The maintenance of probiotic viability during non-refrigerated storage in dry state is still a challenge to functional food industry because the probiotics are live microorganisms and susceptible to environmental stresses, especially the moisture at the ambient storage conditions (Ross 2005; Heidebach 2012).

Coating probiotic bacteria with shellac, a natural polymer, has been shown to enhance the survival of probiotics during gastric transit (Stummer 2010). This study investigates the effect of shellac coating on probiotic microcapsule granules on (i) the moisture uptake and (ii) the survival of probiotics during non-refrigerated storage of probiotic bacteria, *Lactobacillus rhamnosus* GG (LGG).

MATERIALS AND METHODS

Materials LGG was from Laboratorium Voor Microbiologie, Ledeganckstraat, 117 Belgium. Glucose was from Penford Australia Ltd, NSW, Australia. Skim milk powder (SMP HHT) was from Fonterra Australia Pty Ltd, Victoria, Australia. Shellac (Aqualacca 25, 25% total solids (TS)) was provided by Chemacon GmbH, Mainz, Germany. A saturated solution of NaBr (Fluka, Zwijndrecht, Netherlands) was used to create 57% relative humidity (RH) environment during storage.

Sample preparation and characterisation Fresh LGG was cultured and harvested by following similar procedure described in Zhu et al (2013). The LGG concentrate was diluted using de-ionized water and a mixture of skim milk powder: glucose (1:1 ratio) was added and mixed, at room temperature, using a bench-top mixer to produce a paste containing 2% (dry basis) of LGG (Figure 1). The paste was then extruded through a die with a mesh of 0.5 mm holes to form noodle-like extrudates, which were frozen in liquid nitrogen, then freeze dried overnight and broken into small pellets with a length of around 1-2 mm. The pellets were coated with shellac in a Glatt GPCG 2 fluid bed with a Wurster insert. The fluidizing airflow was maintained at 110 m^3/hr and inlet temperature at 40°C. Shellac solution (25% total solids) was fed at 4 ml/min using a peristaltic pump and sprayed using a twin fluid nozzle with air pressure of 2 bar. The ratio of shellac:probiotic pellets was 1:5 (dry solids basis).

Pellets, with or without coating, were characterised for morphology using optical microscopy, moisture sorption behaviour using dynamic sorption system (DVS Series 2000, Surface Measurement Systems Ltd., London, UK) (Ying 2010) and glass transition temperature (Tg) using a rheometer (Physica MCR 301, Anton Paar Germany GmbH) (Ying 2012). The LGG viability of the pellets were tested using the plating method as described in Ying et al. (2012), and samples were taken at time points of 0, 7, 21 and 35 days during storage at 25°C and 57%RH environment, which was created by saturated NaBr solution in hermitically sealed glass desiccators.

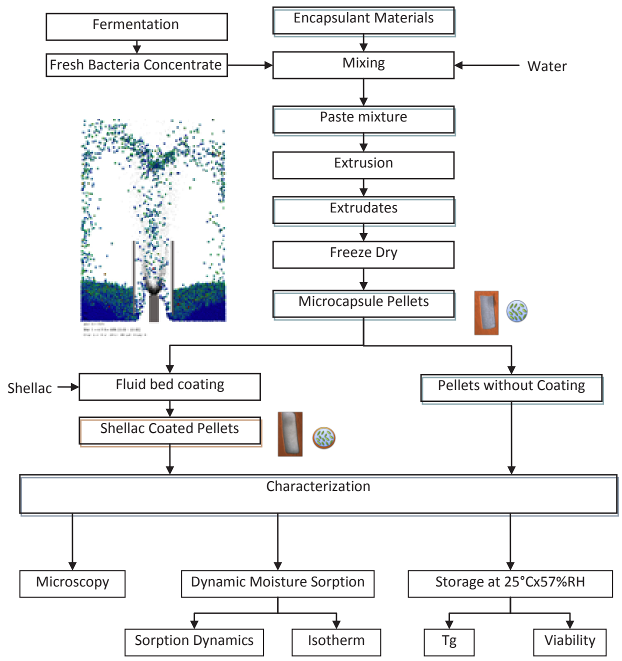

Figure 1 : Sample preparation procedure and characterization flow chart

RESULTS AND DISCUSSIONS

Optical microscopy examination revealed a smooth coating on the surface of shellac coated pellets (Figure 2, inserts). The sharp edges of the original pellets disappeared in the coated pellets.

Moisture uptake of the pellets without coating and that with coating exposed to 57%RH at 25°C is given in Figure 2.

The shellac coated pellets took a much longer time to reach the maximum equilibrium moisture content. The slower rate of moisture uptake of shellac-coated pellets may be expected because of the known effect of shellac on reducing moisture permeability (Phan The 2010).

The shellac coating suppressed the moisture uptake at 57%RH. It is known that shellac itself absorbs moisture (Soradech 2012). However, the measured moisture uptake of shellac-coated pellet was less than the sum of the expected theoretical uptake of the individual pellet and shellac components (Figure 2). There is possibly some interaction of shellac and components and altered assembly of the components in the sample which resulted in lower water binding properties.

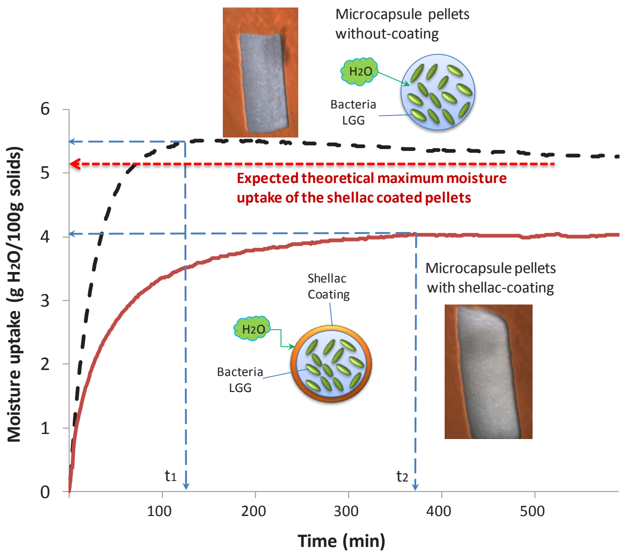

Figure 2 : Dynamic moisture uptake of the freeze dried pellets, without (broken line) and with (solid line) shellac coating, when exposed to 57%RH at 25°C, and the schematic diagrams (the inserts) showing the cross section of the pellets containing LGG. The t_1 and t_2 are the time taken for pellets to absorb moisture to the maximum at 57%RH.

Moisture sorption isotherms of the pellets with and without coating (Figure 3) showed that there is a cross over at RH = 30% (Insert in Figure 3). Therefore one has to consider the effectiveness of coating materials at the desired storage conditions.

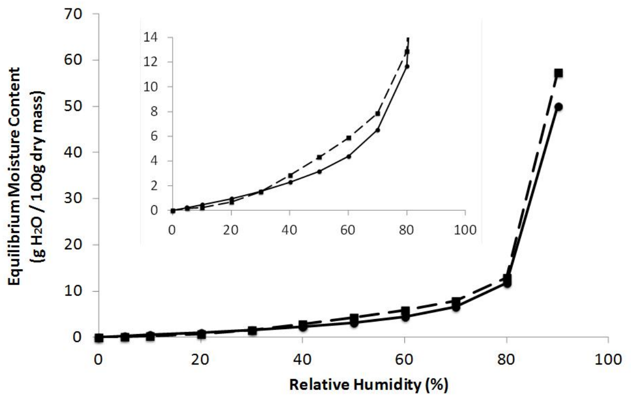

Figure 3 : Moisture sorption isotherms of the pellets without (broken line) and with (solid line) shellac coating. The error is estimated < 2.5%.

Glass transition temperatures of the pellets with and without shellac coating were 67°C and 65°C, respectively, indicating a slight increase in the Tg because of the shellac coating.

Probiotic survival of LGG was significantly improved ($p<0.01$) in the shellac coated pellets after 35 days storage at 25°C and 57%RH (Figure 4). This is attributed to the hindered moisture uptake (Figure 2).

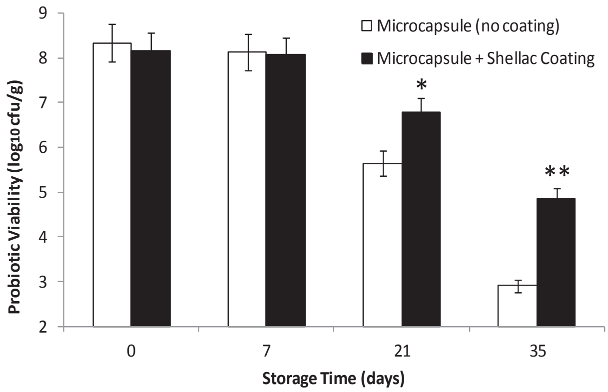

Figure 4 : Viability of LGG in the extruded skim milk - glucose pellets without coating (white) and with shellac coating (black), *($p<0.05$), **($p<0.01$).

CONCLUSIONS

Coating microencapsulated LGG with shellac significantly ($p<0.01$) enhanced the survival of probiotics during non-refrigerated storage (25°C) at intermediate moisture environment (57%RH) over 35 days period. Shellac coating is a promising approach for extending the viability of probiotics. Its use should be examined under specific product storage conditions to confirm its suitability in commercial storage applications.

ACKNOWLEDGMENTS

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