Enhanced Survival of Spray Dried Microencapsulated *Lactobacillus rhamnosus* GG in the Presence of Glucose

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Short title: Glucose improves the storage stability of spray dried LGG powders

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Abstract

The survival of spray dried *Lactobacillus rhamnosus* GG (LGG) preparations encapsulated in whey protein isolate (WPI)-maltodextrin, WPI-maltodextrin-glucose, WPI-inulin, and WPI-inulin-glucose mixtures during storage at 25°C (11-70% relative humidity, RH) was examined. The incorporation of glucose in the drying medium enhanced survival of LGG when powders were stored at 11% RH (i.e. when all formulations were in the glassy state) as well as at 70% RH (i.e. when all formulations were in the non-glassy state). The superiority of glucose-containing formulations for preservation of LGG was also observed during storage at 57% RH despite the fact that glucose-containing formulations were in a non-glassy state while the WPI-inulin based LGG powder was at the glass transition temperature and the WPI-maltodextrin based LGG powder was in a glassy state. These results showed that the type of sugar/carbohydrate in the drying medium had a greater influence on survival of LGG during storage than the maintenance of a glassy state when formulations were stored under similar environmental conditions. However, both the maintenance of a glassy state during storage and the incorporation of glucose in the drying medium were required for improved survival of probiotic powders prepared from freshly prepared cultures. It is suggested that a direct interaction between glucose and cell components, which helps preserve cell functions during drying is a pre-requisite for improved LGG survival during storage.

Practical Significance

The incorporation of glucose into the drying medium prior to spray drying of protein-carbohydrate based LGG formulations improves the survival of LGG during long term storage.
1. Introduction

Probiotics are live micro-organisms that confer health benefits to humans or animals when consumed in sufficient numbers, typically $>10^7$ CFU g$^{-1}$ (FAO/WHO, 2001). Probiotics may be supplied as frozen cultures or dried preparations (Champagne et al. 1991; Ross et al. 2005; Meng et al. 2008). The removal of water during concentration, evaporation and drying causes a stress to the probiotic, and it is necessary to incorporate protective agents which preserve the integrity of membrane and proteins/enzymes during these processes. Many protective agents have been used for preserving the function of microorganisms in the dry state. Sucrose and trehalose have been most used (Crowe et al., 1998), although other sugars (e.g. maltose, fructose) and sugar alcohols (e.g. sorbitol, inositol) also exert protective effects in dried preparations (Leslie et al., 1995; Linders et al., 1997; Carvalho et al., 2002). The protective effect of sugars on membrane integrity and proteins in the dry state has been ascribed to the ability of sugars to bind to polar residues of proteins and form a glass in the dry state. Both these actions of sugars, namely the interactions with membrane components as well as the maintenance of a glassy state, are important for preserving the function of biological components upon dehydration (Sun et al., 1996; Crowe et al., 1998). Studies on liposomes, as a model for membranes, showed that a mixture of hydroxyethyl starch and glucose stabilized liposomes during drying (Crowe et al., 1997).

Dry preparations of probiotics are usually supplied as freeze dried powders. Spray drying of cultures may be used as an alternative drying method. There has been concern that higher temperatures used during spray drying may be detrimental to
bacteria. However this is not the case for certain lactic acid bacteria. For example, similar survival rates were obtained on freeze drying and spray drying of concentrated cultures of *Lactobacillus bulgaricus* (Teixeira et al., 1995). Cellular damage to probiotics may be reduced and the survival of probiotics during drying enhanced through control of drying parameters, specifically lowering outlet temperature of spray dryers (O’Riordan et al., 2001; Ananta et al., 2005) and the incorporation of appropriate carriers into the drying medium prior to drying (Ananta et al., 2005; Crittenden et al., 2006). The addition of sugars to the growth medium also influences the survival of dried probiotic preparations (Carvalho et al., 2004).

Chávez & Ledeboer (2007) examined various combinations of proteins (soy protein isolate, skim milk powder) and carbohydrates (gum Arabic, maltodextrin, lactose, trehalose, sucrose) as carriers for *Bifidobacterium lactis BB12* in dried powders prepared by combined spray drying and vacuum drying. Kurtmann et al. (2009) showed that the nature of the sugar had an overriding influence over the maintenance of the glassy state in freeze dried *Lactobacillus acidophilus* preparations in sucrose and lactose matrices, where survival of the probiotics in a non-glassy sucrose matrix was greater than that in a glassy lactose matrix. However, survival during drying does not relate to survival during storage and further the maintenance of a glassy state alone during storage does not ensure stability of probiotics in the dry state (Chávez & Ledeboer, 2007; Higl et al., 2007; Kurtmann et al., 2009).

The present study investigates the influence of the type of carbohydrates used in combination with protein (whey protein isolate, WPI) in the drying medium on the
storage stability of spray dried *Lactobacillus rhamnosus* GG microcapsules. The encapsulant formulations chosen were (a) 1 WPI : 2 maltodextrin, (b) 1 WPI : 1 maltodextrin : 1 glucose, (c) 1 WPI : 2 inulin and (d) 1 WPI : 1 inulin : 1 glucose mixtures. The viability of spray dried LGG microcapsules during non-refrigerated storage at various RH (11%, 57% and 70%) over an 8-week period was examined. The glass transition temperatures of the encapsulant matrices at various RH were also determined. It was envisaged that these experiments would yield information on the relative importance of the glassy state and the nature of the carbohydrate for preserving LGG viability.

2. Materials and methods

2.1 Materials

The probiotic bacteria, LGG, were produced by fermentation using a reconstituted skim milk based culture medium and an inoculant of commercial freeze dried LGG (Valio Ltd., Helsinki, Finland). The skim milk powder (33% milk protein, 54.8% lactose, 0.8% fat, 7.8% minerals and 3.6% moisture) was purchased from Fonterra Australia Pty Ltd., Victoria, Australia. Yeast extract (Autolysate Yeast 60%) was supplied by AB Mauri, Queensland, Australia. Neutrase (Neutrase 0.8L) and beta-galactosidase were from Novozymes Australia Pty Ltd., New South Wales, Australia. Materials used for encapsulation were whey protein isolate (WPI) from Fonterra Cooperative Group Ltd., Auckland, New Zealand, maltodextrin DE5 (MALTRIN®M040, Grain Processing Corporation, U.S.A.), inulin (Fibruline®, Salkat Australia Pty. Ltd., Victoria, Australia) and glucose (Penfold Australia Ltd., New South Wales, Australia). The formulations used are given in Table 1.
Saturated salt solutions were used to create the desired relative humidity environments. Lithium chloride was obtained from Sigma-Aldrich, Sydney, Australia; magnesium chloride from BDH Chemical, Australia P/L, Australia; sodium bromide from Fluka, Zwijndrecht, The Netherlands and strontium chloride from Univar, Ajax Chemicals, P/L, Sydney, Australia. De Man, Rogosa & Sharpe (MRS) agar for plating was obtained from Oxoid Ltd (Hampshire, England).

2.2 Preparation of the probiotic inoculant

The culture medium was a 200 litre batch of reconstituted skim milk (10% total solids, TS) containing 0.4% (wet weight) yeast extract, 0.05% (wet weight) Neutrase and 0.01% (wet weight) beta-galactosidase which had been incubated at 45°C for 2 hr. The culture medium was adjusted to pH 6.5, and ultra high temperature treated at 140°C for 8 s and cooled to 37°C. An LGG inoculant was prepared from commercial freeze dried LGG (~1 x 10^{12} CFU g^{-1}) grown in the reconstituted skim milk based culture medium. This was prepared by incubation of 0.5 g of commercial freeze dried LGG in 4 litre of reconstituted skim milk based culture medium at 37°C for 20 hr. This inoculant was then added to 200 litre of the culture medium and further incubated at 37°C for another 20 hr, with the pH being adjusted to pH 6.5 every 30 min using 2 M NaOH solution. These conditions have been previously used for culturing of lactic acid bacteria (Geoff Knight, personal communication).

After 20 hr fermentation, the culture, with a cell density of 7.7 x 10^9 CFU mL^{-1}, was cooled to below 10°C by pumping through a stainless steel plate heat exchanger (T4, APV Australia Pty Ltd, VIC, Australia). The culture was first concentrated using a continuous centrifuge (Westfalia Separator, KNA 3-06-0/6 AG, Germany), operated at ~5000 rpm, and then further concentrated using a batch centrifuge (Beckman J6-
HC Centrifuge, Fullerton, California, U.S.A.) operated at ~3000 g (4°C) for 15 min. The final LGG concentrates (6.2 x 10^{11} CFU g^{-1}, 82% moisture), were stored overnight at 4°C prior to encapsulation.

2.3 Preparation of microencapsulated LGG powders

An aqueous dispersion of WPI (8% TS) was prepared by dispersing this ingredient in water (60°C). The dispersion was allowed to hydrate for 30 min, pH-adjusted to 7.5 using 1 M NaOH and heated to 90°C for 10 min. The required carbohydrates (Table 1) were added at 90°C and the mixture was stirred for a further 10 min. The protein-carbohydrate mixture (encapsulant solution) was then cooled to 15°C and the LGG concentrates were added and mixed into the encapsulant mixture using a Silverson mixer (Silverson Machines Ltd., Waterside HP5 1PQ Chesham Bucks, England). The LGG containing mixture was spray dried using a laboratory scale Drytec spray dryer (Drytec Engineering, LLC, USA) operated at inlet and outlet temperatures of 160°C and 65°C respectively. Two independent fermentation and production runs were carried out. Blank encapsulant powders were produced by spray drying the encapsulant solutions without LGG added and used for estimation of glass transition temperature.

2.4 Storage of spray dried probiotics

Spray dried samples were stored in hermetically sealed glass desiccators at 25°C under three relative humidity conditions (11%, 57% and 70% RH) generated by saturated salt solutions of LiCl (11% RH), NaBr (57% RH) and SrCl₂ (70% RH). The viability of LGG in samples was analysed after 1, 2, 3, 4, 6 and 8 weeks storage.

2.5 Characterisation of encapsulant matrices
2.5.1 Measurement of glass transition temperature by rheometry

The glass transition temperature (Tg) of the blank encapsulant formulations which had been stored over silica gel (0.0% RH) or saturated salt solutions of LiCl (11% RH), MgCl$_2$ (33% RH), NaBr (57% RH) and SrCl$_2$ (70% RH), respectively, were measured using rheometry. Water activity was measured at room temperature using a Decagon water activity meter with CX-2 system (Decagon Devices Inc., Pullman, Washington, U.S.A.).

The technique described by Hogan et al. (2010) was adapted for Tg measurement and the glass to rubber transition was detected by a laboratory rheometer (Physica MCR 301, Anton Paar Germany GmbH). A Peltier system PTD 200, a combination of a lower plate (P-PTD 200/56) and a hood (H-PTD 200), was used to control the temperature. To make a measurement, a sample (1 g) of each powder was compressed between the parallel plates (PP50/Q1 profile, diameter 50 mm) with a normal force of 50 N. A small amplitude shear oscillation (0.1 μN m at 25 Hz) was applied in order to promote an even distribution of the powder sample. This approach is analogous to a stress relaxation process, in which the change of normal force (N) was measured at a constant gap between these two plates, following an initial compression to a normal force of 50 N. Normal force was recorded over time while the sample was heated up by the Peltier plate at a rate of 2ºC min$^{-1}$. The temperature range varied from 0 to 120ºC depending on the sample tested. Tg was identified as the onset temperature (ºC) at which a significant decrease in normal force was observed. Data were recorded by the Rheoplus software (3.0×). A triplicate samples were used for each formulation.

2.5.2 Dynamic Vapour Sorption
Water vapour sorption properties of the blank encapsulant matrices were determined at 25°C using a Dynamic Vapour Sorption System (DVS Series 2000, Surface Measurement System Ltd., London, UK). The methodologies are as described in Ying et al. (2010). Briefly, the equilibrium moisture content at relative humidity of 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90% were obtained using the DVS system and isotherms were fitted with Guggenheim-Anderson-De Boer (GAB) model,

\[ \frac{m}{m_0} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w + CKa_w)} \]

Where, \( m \) is the equilibrium moisture content (g of water per 100 g of dry mass), \( m_0 \) is the monolayer water content (g of water per 100 g of dry mass); \( C \) and \( K \) are the GAB parameters.

### 2.6 Enumeration of probiotics

The LGG powders were rehydrated in sterile phosphate buffered saline (PBS, pH 7.2 ± 0.2) in a shaking water bath (Julabo SW23, John Morris Scientific Pty Ltd., Germany) to facilitate the release of probiotics (37°C, 100 rpm shaking rate) from the matrix. After one hour incubation, appropriate peptone dilutions were prepared and the plating was accomplished by a Whitley automatic spiral plater (Don Whitley Scientific Pty Ltd., Shipley, West Yorkshire, England) on solid MRS agar plates. The MRS agar plates were incubated under an anaerobic condition (created by AnaeroGen\textsuperscript{T,M}, Oxoid Ltd., Hampshire, England) at 37 ± 2°C for 48 ± 3 hr (Volkert et al., 2008).

LGG colonies were counted on a spiral plate colony viewer (Spiral System\textsuperscript{T,M}, Spiral systems INC. U.S.A.). The viable bacteria number was expressed in CFU g\textsuperscript{-1} of powder. The logarithmic value of residual LGG counts after a given storage period \( N_t \)
(Log\(_{10}\) CFU g\(^{-1}\)) was fitted with a linear equation (Abe et al., 2009; Chávez & Ledeboer, 2007):

\[ N_t = -kt + N_0 \]

Where, \(k\) is the absolute value of the regression coefficient of each regression line, and \(t\) is the storage period (week). The value \(k\) was defined as the inactivation rate constant (Log\(_{10}\) CFU g\(^{-1}\) week\(^{-1}\)) in each storage condition.

### 3. Results

#### 3.1 Glass transition temperature of encapsulant matrices

Figure 1 shows the glass transition temperature as a function of %RH of the storage environment of the blank encapsulant matrices. As expected, where only one type of carbohydrate was used in combination with WPI, the Tg of the matrix containing the higher molecular weight carbohydrate (maltodextrin) was higher than that of the matrix containing inulin. Also as expected, when part of the maltodextrin or inulin was replaced by glucose, the Tg of the encapsulant matrices was reduced. At 25°C, the 1 WPI : 2 maltodextrin matrix was in a glassy state for all the conditions used in this study for storage of probiotic powders (11%, 57% and 70% RH). The 1 WPI : 1 maltodextrin : 1 glucose matrix was in the glassy state only at 11%RH and in a non-glassy (rubbery) state at 57% and 70% RH at 25°C. The 1 WPI : 2 inulin matrix was in a glassy state at 11% RH, at its Tg at 57% RH and in a rubbery state at 70%RH at 25°C. The 1 WPI : 1 inulin : 1 glucose matrix was in a glassy state at only 11%RH, and in a non-glassy (rubbery) state at 57% and 70% RH (Figure 1).

#### 3.2 Moisture isotherms of encapsulant matrices
Figure 2 shows the moisture sorption isotherms of the matrices. The equilibrium moisture contents (EMC) of the matrices were influenced by the type of carbohydrate in the matrix. At 11% RH, the incorporation of glucose decreased the EMC while at 70% RH, glucose incorporation increased EMC. At 57% RH, the effect of added glucose was dependent on the other carbohydrate in the formulation (Table 2). Partial replacement of maltodextrin or inulin with glucose increased the monolayer water (Table 2). This is likely to be due to the increase of the number of polar sites in the matrices with glucose. However, the water binding energy of these polar sites are not as strong as that without glucose as reflected in the smaller GAB C values (Table 2), which relates to the energy of sorption of the water molecules in the monolayer region (Rizvi, 1994).

3.3 Stability of probiotics during drying and storage

The incorporation of glucose in formulations did not influence the survival of probiotics during drying. However, glucose incorporation improved the LGG viability of the spray dried preparations exposed to 11%, 57% and 70% RH (Table 3). For each formulation, there was the expected trend of greater loss in viability as RH was increased. The major finding was that under the same storage conditions the viability of LGG in both glucose-containing formulations at all points of storage were greater than non-glucose containing formulations. Both the glucose-containing formulations had similar rates of inactivation, which was lower than that of 1 WPI : 2maltodextrin and 1 WPI : 2 inulin formulations (Figure 3).

3.4 Relationship between the nature of sugar, state of matrix and probiotic survival
Figure 4 shows the relationships between the effects of partial substitution of maltodextrin or inulin with glucose in the WPI-carbohydrate matrices and the physical state (i.e. glassy / rubbery) of the matrices under the various storage conditions. The beneficial effects of added glucose were obtained when (a) all formulations were in the glassy state (Figures 4A & 4B), (b) when glucose-containing formulations were in rubbery state, the WPI-inulin formulation was at the glass transition temperature (Figure 4D) and the WPI-maltodextrin formulation was in the glassy state (Figures 4C and 4E) and (c) when all formulations were in the rubbery state (Figure 4F). These results clearly demonstrated that while a glassy state was desirable for survival of LGG, the type of sugar / carbohydrate in the encapsulant matrix had the major effect on the survival of the probiotics rather than the physical state of the matrix when stored under similar environmental conditions.

4. Discussion

The incorporation of glucose in formulations did not influence the survival of probiotics during drying but had an effect on LGG survival during subsequent storage. These results corroborate those of others who also found that although the survival of LGG during spray drying was not significantly affected when different media (reconstituted skim milk (RSM); RSM/polydextrose; RSM-Raftilose P95) were used in formulations, the different media influenced survival of bacteria during long term storage (Ananta et al., 2005). A similar result was reported by Carvalho et al. (2002) where the presence of sugars (fructose, trehalose or sucrose) or sugar alcohols (inositol, sorbitol) improved survival of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage but not during freeze drying.
The glucose-containing formulations in this study, improved storage stability of spray dried LGG microcapsules stored under similar environmental conditions although the glass transition temperature of these preparations was depressed in comparison to those of formulations without glucose. This implies that the environment created by glucose in the LGG microcapsule has a major effect on LGG survival. Our results (Figure 4) clearly demonstrate that both the maintenance of a glassy state during storage and the incorporation of glucose in the drying medium at a non-glassy state are required for improved survival of LGG powders.

The beneficial effects of glucose could be due to the protective effects of sugars on bacterial cells when the water is removed during drying process. It has been suggested that the incorporation of small sugars improves survival of bacteria during drying because of their ability to replace water that is removed from proteins / enzymes within the cells and reduce the membrane phase transition temperature (Leslie et al., 1995; Castro et al., 1997). The results of this work are in line with those of others which show that a glassy state during storage alone is not sufficient for stabilisation of dried bacterial preparations (Ananta et al., 2005; Crowe et al., 1998; Kurtmann et al., 2009). Protectants which preserve the structural integrity of cell membranes, proteins and enzyme functions are required for improving viability during storage of dried probiotic preparations. These results suggest that a pre-requisite for LGG survival in the glassy state is the direct interaction between a low molecular weight sugar and cell components, which helps preserve cell functions during drying and storage. Therefore both the maintenance of a glassy state during storage and the incorporation of glucose or a low molecular weight sugar in the drying medium were required for improved survival of probiotic powders during storage.
Acknowledgements

Authors would like to thank Geoff Knight and Daryl Unthank for assistance in the preparation of probiotic cultures, Wayne Beattie for assisting in fermentation of cultures and spray drying, and Roderick Williams for advice in rheometry.


List of Figure Captions:

Figure 1. Glass transition temperature (Tg) of encapsulant matrices as a function of RH. (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose. Matrices were pre-equilibrated at 25°C to obtain the desired RH.

Figure 2. Moisture sorption isotherms of encapsulant matrices. (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose.

Figure 3. Inactivation rate constant of LGG as a function of relative humidity during storage at 25°C. LGG encapsulated in (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose.

Figure 4. Survival of LGG during storage at 25°C and relative humidity at 11% RH (A and B), 57% RH (C and D) and 70% RH (E and F) over 8 weeks. LGG encapsulated in (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose.
### Table 1. Formulations of LGG microcapsules on a dry weight basis

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LGG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Protein</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LGG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>WPI</td>
<td>maltodextrin</td>
</tr>
<tr>
<td>1 WPI : 2 maltodextrin</td>
<td>2%</td>
<td>32.7%</td>
<td>65.3%</td>
</tr>
<tr>
<td>1 WPI : 1 maltodextrin : 1 glucose</td>
<td>2%</td>
<td>32.7%</td>
<td>32.7%</td>
</tr>
<tr>
<td>1 WPI : 2 inulin</td>
<td>2%</td>
<td>32.7%</td>
<td>65.3%</td>
</tr>
<tr>
<td>1 WPI : 1 inulin : 1 glucose</td>
<td>2%</td>
<td>32.7%</td>
<td>32.7%</td>
</tr>
</tbody>
</table>

<sup>a</sup>LGG was added as a freshly prepared concentrate (18% TS)
Table 2. Equilibrium moisture content (EMC) of the encapsulant matrices at various RH and the calculated GAB parameters

<table>
<thead>
<tr>
<th>Matrices</th>
<th>EMC (g water/g of solids)</th>
<th>GAB Parameters&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11%RH</td>
<td>57%RH</td>
</tr>
<tr>
<td>1 WPI : 2 maltodextrin</td>
<td>4.22</td>
<td>11.14</td>
</tr>
<tr>
<td>1 WPI : 1 maltodextrin : 1 glucose</td>
<td>2.50</td>
<td>9.84</td>
</tr>
<tr>
<td>1 WPI : 2 inulin</td>
<td>3.33</td>
<td>9.99</td>
</tr>
<tr>
<td>1 WPI : 1 inulin : 1 glucose</td>
<td>2.35</td>
<td>10.78</td>
</tr>
</tbody>
</table>

<sup>a</sup> GAB parameters: $m_0$ - monolayer water content; $C$ - relates to heat of sorption of monolayer water; $K$ - relates to heat of sorption of multilayer water.
Table 3. Viability of LGG of the microcapsules stored at 25°C at various RH

<table>
<thead>
<tr>
<th>%RH</th>
<th>Encapsulant</th>
<th>CFU g⁻¹ powder</th>
<th>Storage (week)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>11%</td>
<td>1 WPI : 2 maltodextrin</td>
<td>(3.5±0.8)x10⁸</td>
<td>(9.4±2.7)x10⁷</td>
<td>(2.1±0.4)x10⁶</td>
<td>(1.5±0.2)x10⁶</td>
<td>(8.8±2.3)x10⁵</td>
<td>(5.5±0.3)x10⁵</td>
<td>(4.8±1.3)x10⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI: 1 maltodextrin: 1 glucose</td>
<td>(1.7±0.2)x10⁸</td>
<td>(1.4±0.6)x10⁸</td>
<td>(2.0±0.4)x10⁷</td>
<td>(1.2±0.3)x10⁷</td>
<td>(8.3±0.4)x10⁶</td>
<td>(6.7±1.1)x10⁶</td>
<td>(9.1±2.5)x10⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI : 2 inulin</td>
<td>(1.4±0.1)x10⁸</td>
<td>(4.8±0.4)x10⁷</td>
<td>(2.4±0.2)x10⁶</td>
<td>(1.4±0.1)x10⁶</td>
<td>(1.4±0.1)x10⁶</td>
<td>(9.3±3.1)x10⁵</td>
<td>(7.7±0.4)x10⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI : 1 inulin : 1 glucose</td>
<td>(2.7±0.4)x10⁸</td>
<td>(5.5±1.9)x10⁸</td>
<td>(4.8±0.9)x10⁷</td>
<td>(1.3±0.3)x10⁷</td>
<td>(2.4±0.1)x10⁷</td>
<td>(2.9±1.1)x10⁷</td>
<td>(1.2±0.0)x10⁷</td>
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<td></td>
</tr>
<tr>
<td>57%</td>
<td>1 WPI : 2 maltodextrin</td>
<td>(3.5±0.8)x10⁸</td>
<td>(7.4±7.6)x10⁷</td>
<td>(6.6±1.7)x10⁵</td>
<td>(4.1±0.8)x10⁴</td>
<td>(1.1±0.4)x10³</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI: 1 maltodextrin: 1 glucose</td>
<td>(1.7±0.2)x10⁸</td>
<td>(1.4±0.1)x10⁸</td>
<td>(4.1±1.1)x10⁷</td>
<td>(2.0±0.2)x10⁶</td>
<td>(1.2±0.1)x10⁵</td>
<td>(1.0±0.0)x10³</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI : 2 inulin</td>
<td>(1.4±0.1)x10⁸</td>
<td>(4.3±0.8)x10⁷</td>
<td>(2.7±0.6)x10⁶</td>
<td>(1.7±0.3)x10⁵</td>
<td>(8.1±1.6)x10³</td>
<td>ND</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI : 1 inulin : 1 glucose</td>
<td>(2.7±0.4)x10⁸</td>
<td>(3.4±0.7)x10⁸</td>
<td>(6.9±0.9)x10⁷</td>
<td>(3.0±0.1)x10⁶</td>
<td>(1.8±0.1)x10⁵</td>
<td>(1.0±0.0)x10³</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70%</td>
<td>1 WPI : 2 maltodextrin</td>
<td>(3.5±0.8)x10⁸</td>
<td>(3.3±0.9)x10⁷</td>
<td>(1.6±0.9)x10⁵</td>
<td>(5.8±1.3)x10³</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI: 1 maltodextrin: 1 glucose</td>
<td>(1.7±0.2)x10⁸</td>
<td>(1.3±0.2)x10⁸</td>
<td>(3.8±2.8)x10⁷</td>
<td>(1.4±0.1)x10⁵</td>
<td>(1.2±1.3)x10³</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 WPI : 2 inulin</td>
<td>(1.4±0.1)x10⁸</td>
<td>(4.0±0.6)x10⁷</td>
<td>(5.8±1.3)x10⁵</td>
<td>(1.1±0.1)x10⁴</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1 WPI : 1 inulin : 1 glucose</td>
<td>(2.7±0.4)x10⁸</td>
<td>(2.8±0.5)x10⁸</td>
<td>(2.1±0.4)x10⁷</td>
<td>(2.1±0.5)x10⁵</td>
<td>(1.2±0.0)x10³</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

ND – non detectable (<10³ CFU g⁻¹ powder)
Figure 1. Glass transition temperature (Tg) of encapsulant matrices as a function of RH. (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose. Matrices were pre-equilibrated at 25°C to obtain the desired RH.
Figure 2. Moisture sorption isotherms of encapsulant matrices. (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose.
Figure 3. Inactivation rate constant of LGG as a function of relative humidity during storage at 25°C. LGG encapsulated in (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (△) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose.
Figure 4. Survival of LGG during storage at 25°C and relative humidity at 11% RH (A and B), 57% RH (C and D) and 70% RH (E and F) over 8 weeks. LGG encapsulated in (□) 1 WPI : 2 maltodextrin, (■) 1 WPI : 1 maltodextrin : 1 glucose, (∆) 1 WPI : 2 inulin, (▲) 1 WPI : 1 inulin : 1 glucose.