The Viability of Lactic Acid Bacteria and *Bifidobacterium bifidum* in Yoghurt Powder During Storage†

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ABSTRACT

The purpose of this research was to investigate the survival of three different species of lactic acid bacteria and Bifidobacterium bifidum in yoghurt powder during 4 weeks of storage at room and refrigerator temperatures. Fresh yoghurt was prepared from 42.9% (w/w) cow milk, 42.9% (w/w) goat milk, 7.0% (w/w) skim-milk powder, 5.0% (w/w) sugar, 0.2% (w/w) carrageenan, 1.0% (w/w) yoghurt starter culture that was composed of Streptococcus thermophilus and Lactobacillus bulgaricus, 0.5% (w/w) Lactobacillus acidophilus and 0.5% (w/w) Bifidobacterium bifidum and incubated at 42±1°C until the pH of the yoghurt reached a value of 4.6. The fresh yoghurt was dried using a spray drier, followed by packing in PET/PP/Al or nylon/PE packaging. The yoghurt powder was stored at either room or chilled temperature and analyzed every 2 weeks for its chemical and microbial properties. The data showed that lactic acid bacteria and B. bifidum were significantly reduced for up to 4.65 log cfu/g after the drying process. Further reduction in the number of these microorganisms mainly occurred within the first 2 weeks of storage, particularly for B. bifidum. Keeping the yoghurt powder at low storage temperature generally improved the survival of the target microorganisms. Except for L. bulgaricus, the survival of other studied microorganisms was slightly better in PET/PP/Al compared to those in nylon/PE. The pH of the yoghurt powder did not significantly change during the storage period whereas the water activity and moisture content of the yoghurt powder packed in the nylon/PE increased during storage, particularly when the powder was stored at ambient temperature.

Key words: Spray-dried yoghurt, Yoghurt starter culture, Probiotic bacteria, Storage viability

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INTRODUCTION

The term 'probiotic' generally refers to a definition given by Fuller in 1989 that stated probiotic as live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance (Anal and Singh, 2007). Since the microorganisms could positively affect the host's health, the production and consumption of the food products supplemented with these friendly microorganisms have increased dramatically in the past two decades (Mattila-Sandholm et al., 2002). Generally, the food medium that is used to deliver the microorganisms is yoghurt or fermented milk. However, a better understanding of the microorganism characteristics has led different researchers to incorporate the organisms in other food products, including soft, semi-hard and hard cheeses, ice cream, milk powder, fruit products, frozen dairy desserts as well as oat-based products (Alamprese et al., 2002; Mårtensson et al., 2002; Betoret et al., 2003; Akin et al., 2007; Anal and Singh, 2007).

Yoghurt is the most popular fermented milk in the world. This is partly attributed to many desirable effects of the product that are readily accepted by consumers and also due to the image of the product as 'healthy' (Lourens-Hattingh and Viljoen, 2001). The popularity of yoghurt has increased significantly in the last few years because of the incorporation of the probiotic microorganisms into the product that gives an extra nutritional-physiological value. The most popular probiotic bacteria that are added to the yoghurt are *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and the product is called as bio-yoghurt (Lourens-Hattingh and Viljoen, 2001).

To achieve its therapeutic value, it is suggested that the bio-yoghurt should be consumed for more than 100 g per day, containing viable probiotic cells of more than 10^6-10^7 cfu/ml (Lourens-Hattingh and Viljoen, 2001; Akin et al., 2007). However, some papers cited a microbial number of more than 10^9 cells per daily-ingested dose (Prado et al., 2008). To reach this high microbial number, different researchers have investigated the survival of the probiotic in yoghurt (Dave and Shah, 1997a, 1997b and 1997c; Shah and Lankaputhra, 1997; Donkor et al., 2006, 2007). However, there is only a few reported study about the viability of probiotic microorganisms in yoghurt powder.

Yoghurt powder is another yoghurt product that has a benefit of being stable and dry and occupies small volume (Corcoran et al., 2004). The technology of spray drying is the main technological process that is applied for milk and milk products due to its economical process, high production rates and low operating costs (Corcoran et al., 2004). To dry probiotic bacteria using this method, there is a challenge to maintain the culture viability due to high processing temperatures encountered during the process (Stanton et al., 2003). Several approaches that have been proposed to overcome microbial inactivation during drying and subsequent storage period include addition of protective agents such as prebiotics, pH adjustment, microbial encapsulation and sub-lethal pretreatments (O'Riordan et al., 2001; Stanton et al., 2003; Corcoran et al., 2004; Saarela et al., 2004). In this study, the survival of yoghurt bacteria and two probiotic cultures of *L. acidophilus* and *B. bifidum* in yoghurt powder produced by a pilot plant spray

drier was assessed. Results from this study were aimed to understand whether the yoghurt medium could support the viability of the target microorganisms during the process and subsequent storage period at different storage temperatures and packaging materials.

MATERIALS AND METHODS

Microbial cultures

Freeze-dried probiotic cultures of *L. acidophilus* (LA-5) and *B. bifidum* and yoghurt starter cultures (a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) were obtained from CHR-Hansen A/S (USA). These cultures were stored at -18°C until use in any experiment.

Yoghurt production

Basic yoghurt formula was prepared from 42.9% (w/w) cow milk, 42.9% (w/w) goat milk, 7.0% (w/w) skim-milk powder, 5.0% (w/w) sugar and 0.2% (w/w) carrageenan. After thorough mixing of the milk solution, the milk was heated at 72±1°C for 15 s, followed by an immediate cooling to a temperature around 45°C. At this temperature, 1.0% (w/w) yoghurt cultures, 0.5% (w/w) *L. acidophilus* and 0.5% (w/w) *B. bifidum* were incorporated into the milk solution. The milk was then incubated at 43±1°C for up to 6 h until the yoghurt would have a pH value of 4.6. To reduce the yoghurt starter cultures activity, the yoghurt was kept at 4°C until the yoghurt was dried in a spray drier.

Yoghurt powder production

Before drying yoghurt in a spray drier, the total soluble solid of the yoghurt which was around 23.23±1.65% Brix was adjusted to 25% Brix, using a 25% (w/v) maltodextrin solution (Master, 1991). The yoghurt was dried in a spray drier model SDE 50, manufactured by J.C. Machinery and Civil Work Co., Ltd, Thailand. The type of atomizer used was a nozzle atomizer with a length of 44 cm together with an atomizer pressure at 15 psi, a co-current air flow and an inlet temperature of 180°C. Since an outlet air temperature of lower than 60°C would not produce a dried powder and applying higher outlet temperatures of more than 90°C would produce a powder with lower physical quality due to browning reaction (Kim and Bhowmik 1990; To and Etzel, 1997), the outlet temperature applied in this study was 80±2°C. The final yoghurt product was packed in two different packaging materials, including laminated polyethylene tetraphthalate/ polypropylene/aluminium (PET/PP/Al) and laminated nylon/polyethylene (nylon/ PE), and two different storage temperatures of either room or refrigerated temperature for 4 weeks. During the storage period, representative yoghurt samples were analyzed for their microbial and chemical qualities.

Microbiological analysis

Yoghurt or yoghurt powder samples were diluted, using 10-fold serial dilution in Maximum Recovery Diluent (MRD, Oxoid, England) before being pour-plated

in different selective media. The enumeration of *S. thermophilus* was carried out, using M-17 agar (Merck, Germany) and aerobically incubated at 37°C for 48 h (Dave and Shah, 1996; Tharmaraj and Shah, 2003). For *L. acidophilus*, Reinforced Clostridial Agar (RCA, Oxoid, England) and an anaerobic incubation condition at 37°C for 48 h were applied. The viable number of *L. bulgaricus* was determined by deducting the number of microorganisms in tomato juice agar (Oxoid, England) that was incubated anaerobically at 37°C for 48 h with the result from the RCA enumeration whereas the number of *B. bifidum* was revealed by subtracting the number of microorganisms growing in homofermentative and heterofermentative differential Agar (McDonald et al., 1987), which was incubated anaerobically at 37°C for 72 h, with the microbial number from the RCA count.

Chemical analysis

For pH of yoghurt samples, it was determined using a pH-meter (Consort C-830 CE, Belgium) after diluting 1.0 g of the powder in 10 ml of distilled water. Water activity (a_w) of the yoghurt powder was measured by an a_w-meter (Series 3 Aqualab, USA). The measurement of the yoghurt powder moisture content was carried out by drying 1±0.1 g of yoghurt powder in a hot-air oven (Memmert, Germany) at 100±1°C for 4 h. The moisture content was then calculated, using the following equation (AOAC, 2000):

$$\text{Moisture content (\%)} = \frac{\text{loss of sample weight during drying}}{\text{intial sample weight}} \text{ X } 100$$

RESULTS AND DISCUSSION

Results from microbial analysis showed that the number of yoghurt and probiotic bacteria in the fresh yoghurt was higher than 7.0 log cfu/ml, except for the *L. bulgaricus*, which was in the order of 6.06±0.12 log cfu/ml. The viable number of *S. thermophilus* that was 8.38±0.24 log cfu/ml was in accordance with the suggestion level for the number of yoghurt cultures in fermented milks (Adams and Moss, 2000). This number was also one log cycle higher than the one reported by Dave and Shah (1997a). On the other hand, the number of *L. bulgaricus* was one log cycle lower than the one found by Dave and Shah (1997a). Discrepancy in the number of the yoghurt bacteria could be affected by different inoculation levels of the freeze-dried cultures and different compositions of the basic yoghurt formula, in which this study used goat milk as part of the raw material. For the level of the probiotic bacteria, their numbers were higher than the minimum level suggested by Vinderola et al., (2000) and Mårtensson et al., (2002).

The survival of the studied microorganisms was mainly affected during a drying process. Even though a lower outlet temperature was used in this study compared to the one that was used by Corcoran et al., (2004), which was 85 to 95°C, the survival of the four microorganisms in the yoghurt powder was still low. Survival rates of 44.51%, 50.83%, 47.43% and 47.75% were recorded in *S. thermophilus, L. bulgaricus, L. acidophilus* and *B. bifidum*, respectively. This finding could be affected by different media used in different studies. The research

by Corcoran et al., (2004) used a medium of reconstituted skim milk whereas the medium in this study was yoghurt that had a pH value lower than the reconstituted skim milk. Saarela et al., (2004) reported that tolerance to low pH conditions was dependent on microbial strains.

During storage at room and chilled temperatures, the microbial survival was shown to be better at lower storage temperature, particularly for *B. bifidum* (Figs. 1 and 2). Higher survival rates of lactobacilli and bifidobacteria at lower storage temperatures had also been reported by Corcoran et al., (2004) and Simpson et al., (2005). During 4 weeks of storage period, the viability loss of the target microorganisms mainly occurred during the first two weeks of the storage. This could be due to cell membrane damage that occurred during the drying process (Stanton et al., 2003), causing the microorganisms to have difficulty in maintaining their viability during storage at low water activity. After 4 weeks of storage, most of the microorganisms could maintain 70% of their survival rates, except for *B. bifidum*. The low survival rate of the bifidobacteria could be affected by the sensitivity of the organism towards oxygen and heat treatment (Simpson et al., 2005).

With different packaging materials, the data did not show any significant difference between the use of PET/PP/Al and nylon/PE, except for the *B. bifidum* that was stored at refrigerator temperature (Fig. 2). This result could be caused by different air permeability rates of the two packaging materials. It was found that the PET/PP/Al had an air permeability rate of 7.34×10^{-6} $cm^3/m^2.d.Pa$, while the nylon/PE had a rate of 1.15×10^{-3} $cm^3/m^2.d.Pa$. A higher air permeability rate of the latter packaging material should have negatively affected the survival of the *Bifidobacterium*. A research result by Simpson et al. (2005) showed that the tolerance of different *Bifidobacterium* spp. towards oxygen was highly dependent on microbial strains. The study also showed that the tolerance of *B. bifidum* NCMB 795 towards oxygen was categorized as low.

During 4-week storage of the yoghurt powder, the pH of the powder was within 3.82-3.89 (Fig. 3). Even though the analysis results showed some fluctuation in the yoghurt pH values, the discrepancies were not high enough to show any significant effect on the overall chemical characteristic of the product. All the yoghurt powders had a water activity below 0.2 and a moisture content below 2.52% throughout the storage period (Figs. 4 and 5). The low moisture content of the yoghurt powder is important, since a maximum moisture content of 4% has been cited by Corcoran et al., (2004) and Simpson et al., (2005). Muir and Banks (2000) stated that if the moisture content of dried milk products was more than 4%, the deterioration of the product

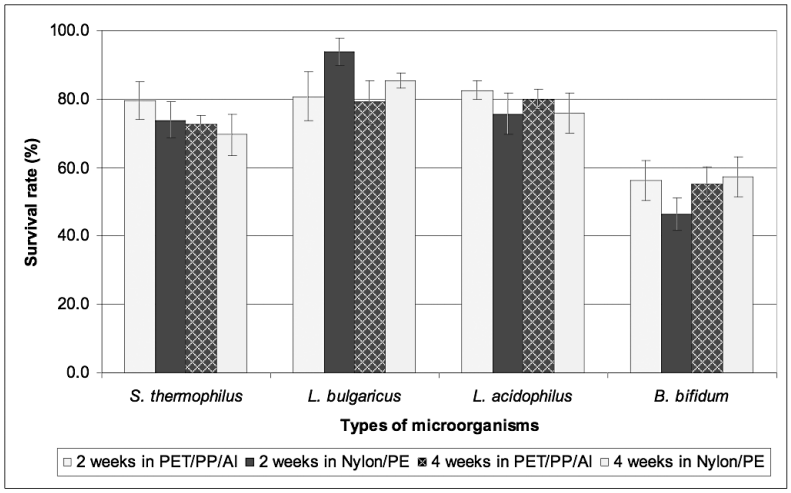

Figure 1. The survival of lactic acid bacteria and *Bifidobacterium bifidum* during room temperature storage in different packaging materials.

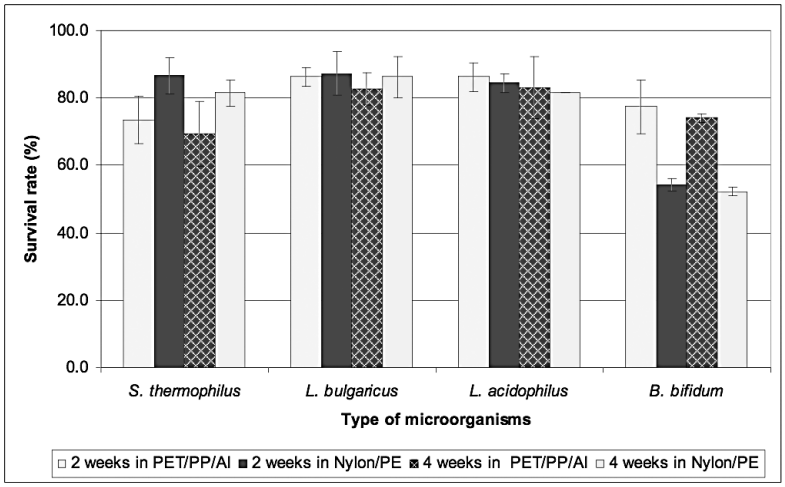

Figure 2. The survival of lactic acid bacteria and *Bifidobacterium bifidum* during storage at 4°C in different packaging materials.

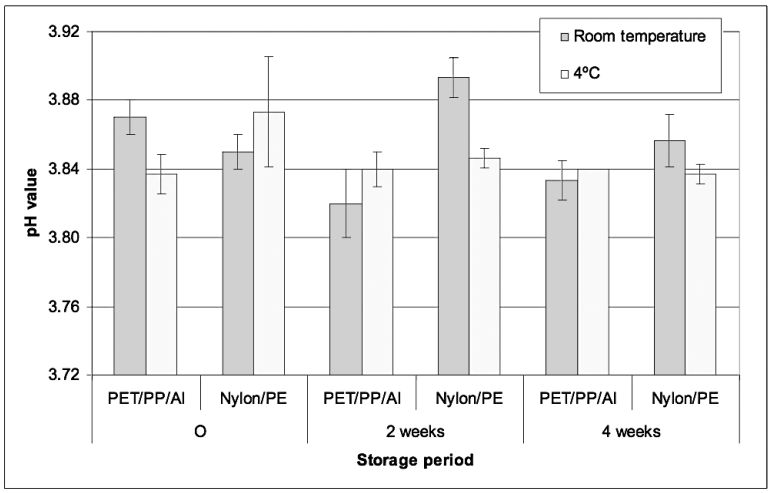

Figure 3. pH values of yoghurt powder containing probiotic bacteria during storage in different packaging materials and storage temperatures.

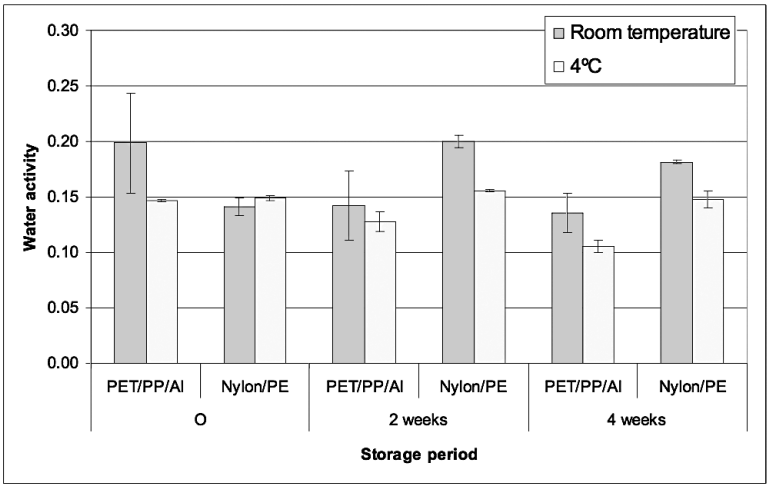

Figure 4. Water activity of yoghurt powder containing probiotic bacteria during storage in different packaging materials and storage temperatures.

would be started. The moisture content of the yoghurt powder in this study was influenced by the packaging materials used to keep the product. At longer storage period, the moisture content of the yoghurt powder had a tendency to be increased, especially when it was stored in the nylon/PE packaging at room temperature. This could be caused by the air permeability rate of the nylon/PE that was higher than that of the PET/PP/Al. Therefore, there is a need to consider a good packaging material to keep the yoghurt powder, especially when it is stored at elevated temperature for a long storage period.

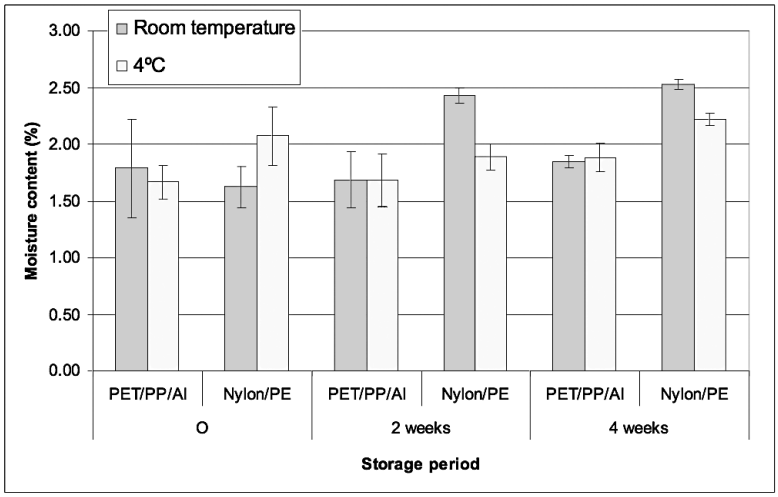

Figure 5. Moisture contents (%) of yoghurt powder containing probiotic bacteria during storage in different packaging materials and storage temperatures.

CONCLUSION

Data from this research showed that yoghurt powder could be an alternative medium to deliver probiotic microorganisms to health-conscious people. The main technological challenge of this product is to maintain a high viability rate of the beneficial microorganisms during drying. An improvement of the microorganism viability by an incorporation of protective agents, pH adjustment or heat-stress adaptation needs to be studied further to understand their potential application in the food industries. Keeping the yoghurt powder at low storage temperature would enhance the survival of the probiotic bacteria during the storage period.

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