Encapsulation of probiotics for cereal bars

Champagne Claude P., Raymond Y., Guertin N.
Food Res & Development Centre, Agriculture & Agri-food Canada, St-Hyacinthe, QC Canada

INTRODUCTION

Cereal products have been an important food matrix for the delivery of functional ingredients. Indeed, cereal-based functional foods are typically enriched with fibres and omega 3 oils. In contrast with dairy products, however, there are only a few examples of enrichment with probiotic cultures. The challenges in the addition of probiotic bacteria to cereal products are: 1) heating (and sometimes freezing) during processing, 2) detrimental levels of moisture and oxygen during storage. Nutrient bars have been proposed as cereal-based matrices for probiotics, but viability issues occur during storage (Saarela et al., 2006). Strategies to avoid some of these problems are: 1) use of thermostable *Bacillus* sp. probiotics, 2) addition of probiotics after heating and 3) micro-encapsulation (ME) (Altamirano-Fortoul, 2012; Siuta-Cruce & Goulet, 2001). This study reports on ME.

Most studies on ME for probiotics in foods have involved cell entrapment in alginate gels (Burgain et al., 2011). However, spray-coating (SC) technology (Durand & Panes, 2003) is increasing commercially. SC is difficult to carry out, and an easier approach is inclusion into fat matrices, which could be achieved by spray-chilling technology. In this study we report on the combination of these various approaches.

MATERIALS AND METHODS

Cultures used *Lactobacillus rhamnosus* R0011 (Institut Rosell-Lallemand, Canada) was used in the free-cell (FC) and microencapsulated by SC. Chocolate particles having 20% of FC or SC powders were also prepared. Melted chocolate was cooled to 40°C, blended with the bacterial powers and pumped so that droplets solidified on a cold stainless steel plate. Some cultures were prepared in alginate beads as described by Champagne *et al* (2012).

Cereal bar production Ingredients were: 150 g rolled oats flake, 78 g sesame seeds, 30 g poppy seeds, 32 g linseed, 50 g cranberries, 42 g almond pieces, 178 g honey. Probiotics were either 28 g (5%) of chocolate chips or 5.6 g (1%) of commercial FC or SC powders.

The ingredients were mixed together except for the honey and chocolate particles (when needed). The honey was heated to 138°C, poured over the dry ingredients and stirred. The probiotic FC and SC powders were blended with the cereal grains prior to honey addition, while the chocolate particles were added 5 minutes after the addition of honey. The mixture was poured into a pan and pressed. After cooling to 4°C, cereal bars were cut, packaged under nitrogen and stored at room temperature.

Analyses *CFU*. A cereal bar (25 g) was added to 225 mL of a rehydration solution (peptone 0.1%, Na ascorbate 0.1%, cysteine 0.05%, Tween 0.1%) at 48°C in a sterile jar and homogenized using a mechanical blender. The homogenate was incubated at 37°C for 15 minutes and subsequently diluted in 0.1% peptone prior to plating on MRS agar (48 h/37°C). *Flow cytometry (FCM)*. After the rehydration and homogenization process carried out for CFU analyses, samples were diluted in NaCl 0.85% to obtain cell suspensions having approximately 10^6 bacteria/mL. Coloration was made with Invitrogen Live Dead Kit on a Accuri C6 flow cytometer with the threshold filter FL1 (530/30) at 1000 and FL2 (580/40) at 600, at slow speed. *Water activity* (a_w) was assessed with an Aqualab CX2 unit.

RESULTS AND DISCUSSION

Survival to chocolate particle production
The FC and SC products were from commercial origin but the probiotic-carrying chocolate particles were prepared for this study. The incorporation of probiotics into chocolate resulted into a slight reduction in viable counts (Table 1). There were no differences between the FC and SC cultures with respect to survival to addition to chocolate. Plating and FCM results were similar. The high survival rate makes chocolate a commercially attractive delivery matrix, which is in line with data from Possemeirs et al. (2010).

Table 1. Viability loss (log_{10}/g) of L. rhamnosus during the production of the chocolate particle

Chocolate Particle	CFU	FCM
Free cell	0.20	0.14
Spray-coated	0.16	0.30

Survival to cereal bar production The free cells had a lower survival level than did the SC product (Table 2). However, addition of the FC to chocolate improved viability to cereal bar production. It remains to be ascertained to what extent this is linked to the later addition of the probiotic chocolate

particle in the process.

Table 2. Viability loss (in log_{10}/g) of L. rhamnosus R0011 during the production of cereal bars.

Culture	CFU	FCM
Free cell	0.27	0.22
Free cell in chocolate	0	0
Spray-coated	0.03	0.05
Spray-coated in chocolate	0.03	0

Survival during storage ME in air-dried alginate protected cells during addition to chocolate, but was ineffective in protecting probiotics during storage (data not shown).

With FC, storage of the cereal bars at 25°C resulted in almost 1 log loss in CFU in only 4 weeks (Figure 1). Microencapsulation by SC improved stability during storage. With FC, addition to chocolate delayed the loss in CFU, but had no benefit with the FC culture (Figure 1).

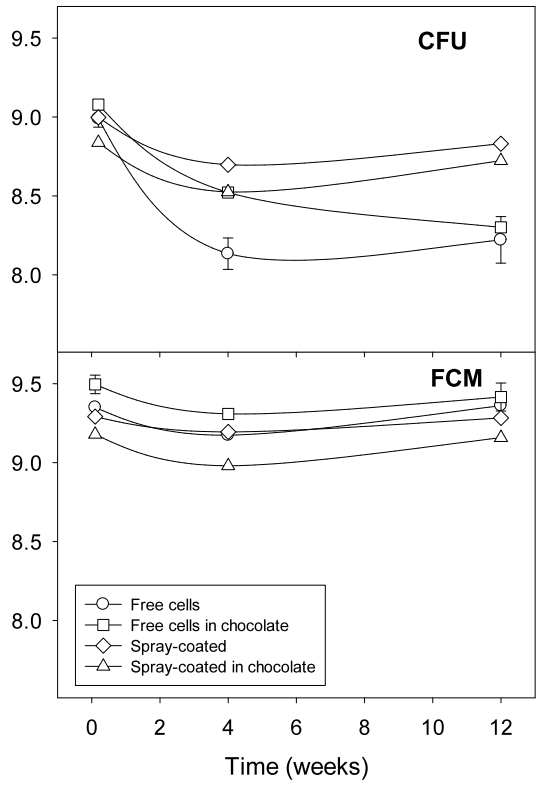

Figure 1. Effect of encapsulation technologies on viable counts of L. rhamnosus R0011 during the storage of cereal bars at 25°C, as assessed by plating (CFU) or flow cytometry (FCW).

Viability assessments by FCM resulted in higher values (Figure 1), which suggests that losses in CFU during storage were not mainly linked to damages to the cell membrane. When the cultures themselves were stored at 25°C, there were also viability losses, but they differed from those in the cereal bar. This is potentially due to a_w. The a_w of the cereal bars was of 0.48, while those of the chocolate-based cultures and the dry cultures were of 0.32 and 0.15 respectively. As a rule, the higher a_w, the lower is stability of probiotic bacteria during storage. Encapsulation by SC or in chocolate probably delayed the hydration of the cultures from the moisture in the cereal bar.

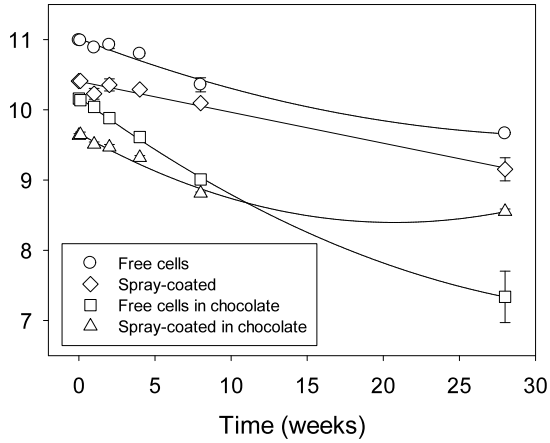

Figure 2. Effect of encapsulation technologies on viable counts of *L. rhamnosus* R0011 during the storage of the cultures themselves (not in cereal bars) at 25°C.

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